

**The Implications of Forager Behaviour for Social Organisation in a Socially
Polymorphic Carpenter Bee (*Xylocopa virginica*)**

by

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Abstract

In social Hymenoptera, the division of labour is a major step in the evolution of sociality. Bees, which express many different kinds of sociality, can be classified according to how individuals share or do not share foraging and reproductive activities (Michener, 1974). The large carpenter bee, *Xylocopa virginica*, lives in populations with both solitary and social nests. In social nests, reproduction is controlled by the dominant female, who does all of her own foraging and egg-laying, while the subordinates guard the nest only. This study examined foraging behaviour as a way to classify the social hierarchy. Individual females were marked, measured and intensely observed for the foraging season. It was found that a large number of subordinates forage and likely obtain more reproductive fitness than previously thought. The dominance hierarchy is very likely a social queue, in which bees take turns foraging and egg-laying.

Acknowledgements

I would like to dedicate this thesis to myself. While at first this may appear a slightly conceited dedication – when I looked back at the monstrosity of this project and thought of all that I have accomplished and had been through, there seemed to be a no more fitting dedicatee, than me.

Doing a Master's degree is an interesting and unique decision mostly because in addition to becoming a “real” scientist, it is also a fantastic way to learn a lot about oneself. Take me for example. I learned that science is a language that takes time to speak properly, that being an MSc student doesn't make you a lot of money, and that with a little dedication and a little help from the right people – you can achieve the highly improbable. Perhaps most importantly of all these things, I learned that I am not unlike the very bees I studied and grew to love, whom, while solitary at times – seem to be most at home in a social group. That being said, this seems like a fitting time to comment on my colleagues and friends that make up my social nest.

Thank you very much to my supervisor, Dr. Richards (clearly the dominant bee), whom, without her guidance and support I would be completely lost. Although sometimes your feedback was a *bit* thorough for my taste, I cannot deny that I have an immense respect for you both as a scientist and as a person.

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“Because we separate like ripples on a blank shore”

-Radiohead

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Introduction

The evolution of sociality in insects

Animals have evolved to live in groups because the gains of living within a group outweigh the losses of living alone (Alexander, 1974). Explanation of why this occurs poses problems in some animal groups in which the needs of the individual and the group do not appear to be equal. One such problem concerns the sterile individuals of highly social or eusocial insect colonies and is addressed in most major publications concerning social evolution (Michener and Lin 1972; Griffin and West 2002; Eberherd, 1972; Andersson 1984). At first it was troubling to imagine not only why individuals would give up their chance at reproduction to raise another's offspring, but also how this altruistic behaviour could evolve. This dilemma was addressed by Hamilton (1964), who argued that if helpers shared enough of their genes with a relative, then helping that relative to raise its offspring would raise the inclusive fitness of the helper. According to Hamilton's rule, a helper should help when b , the number of related brood raised, multiplied by r , the relatedness of the helper to those related brood, is greater than r_0 , the helper's relatedness to its own offspring, multiplied by c , the number of offspring that the helper gives up in order to raise those related brood ($rb > r_0c$). In this way, altruism can evolve in groups in which relatedness is high, which is the case for most eusocial insects.

The theory of inclusive fitness has been used to explain many other phenomena as well, including the evolution of sociality in general. This theory has been challenged on the grounds that competition between relatives can reduce the benefits of altruism (Griffin and West, 2002). Another major criticism arises from the observation that some

insect colonies accept unrelated individuals of the same species. In this way, social behaviour increases the fitness of all individuals in the colony, regardless of relatedness (mutualism), and may contribute to the evolution of sociality in at least some insect species (Lin and Michener, 1972).

Another mechanism that may help explain the evolution of sociality is parental manipulation, in which the mother manipulates her offspring into raising the mother's offspring instead of raising their own (Michener and Brothers, 1974; Andersson, 1984). This may be accomplished through physical means, such as eating the offspring's eggs or by behavioural or chemical control. Regardless of the method of manipulation, the mother's offspring are controlled in such a manner that helping the parent is more beneficial than leaving the nest to reproduce solitarily. In this way being forced to stay in the colony may have played a part in the evolution of sociality. The major conclusion concerning the evolution of sociality, is that social insects represent the most diverse kind of societies, and may be able to evolve sociality through the number of different mechanisms outlined above (Alexander 1974; Andersson, 1984; Lin and Michener, 1972).

Few animal groups exist in which all individuals share reproduction equally or one individual controls all reproduction. Most groups lie somewhere in between these extremes, and how this reproduction is split within the group is addressed by reproductive skew theory (Keller and Reeve 1994). A "high-skew" group has one or a few individuals that monopolize most of the reproductive output, while a "low-skew" group has more evenly distributed reproduction (Keller and Reeve 1994; Waibel *et al.* 2006). Eusocial

insect colonies represent an example of high-skew groups, in that the queen monopolizes most, if not all, of the reproductive output of the colony.

In many animal groups, reproductive output is typically controlled by one individual (dominant), while the others (subordinates) receive fewer reproductive opportunities. Provided that the dominant receives some benefit for allowing the subordinate to remain in the group (e.g. increased protection from predators and conspecifics), dominants should allow subordinates some degree of reproductive output. This is called a staying incentive (Reeve and Emlen, 2000) and represents a transactional model of reproductive skew theory (Langer *et al.* 2004). According to this model, whether or not a subordinate remains in the group depends on the probability that a subordinate can raise more offspring alone than in the group, the genetic relatedness of the group, and the subordinate's fighting ability. Subordinates should stay in the group if their reproductive fitness is higher in the group than would be possible alone. If the subordinate can raise more offspring alone, however, leaving would allow the subordinate to maximize its reproductive fitness and incur no costs from the dominant. If the group is highly related, subordinates should stay since they get direct reproductive fitness from raising their own offspring, with the added benefit of indirect fitness accrued through the dominant's offspring. Finally, if a subordinate has a high probability of usurping the dominant, the dominant may offer an increased staying incentive to prevent being overthrown (peace incentive), which increases the likelihood that the subordinates stay within the group (Keller and Reeve, 1994).

In some groups the dominant is unable to fully control reproduction. In this case, both the dominant and the subordinate must increase their shares of reproduction through individual effort. These groups fit into the tug-of-war model, as individuals are more or less in control of their own reproductive output and compete with one another to maximize individual reproductive fitness (Langer *et al.* 2004).

Reproductive skew models are helpful since they provide a general framework in which hypotheses concerning group evolution can be tested experimentally (Keller and Reeve 1994; Reeve and Emlem, 2000; Langer *et al.* 2004). By using these models, scientists can address major questions about the evolution of sociality in all animals.

Sociality in bees

Among the social insects, few groups are as useful to the study of social evolution as bees, simply because they exhibit all kinds of sociality from solitary behaviour to advanced eusociality (Michener, 1974; Michener 1969; Sakagami, 1960).

Most species of bees are solitary, meaning that a female constructs her own nest and provisions her brood alone with little contact with other individuals. A minority of bee species are social, a term which refers to bees that live in groups for at least part of their adult lives. Sociality in bees is subdivided into five different categories. Arguably the most basic form of sociality is subsociality, which is characterized by a single female providing parental care to her offspring during the larval and/or pupal stages (Tallamy and Wood, 1986; Michener 1974). The difference between subsocial and solitary bees is that the former have behavioural contact with their offspring before they emerge as

adults, which the female may feed or protect, while in the latter parental care is concluded once the egg and provision mass have been sealed in a brood cell.

The most basic form of sociality that involves two or more adult females is communal behaviour, in which bees provision and lay their own eggs within a shared nest. In communal nests there is no reproductive skew as females are essentially solitary, with the exception that they live together. Communal nests in which females cooperatively construct and provision brood cells are termed quasisocial. Nests in which there is some degree of reproductive skew, division of labour and cooperative care are called semisocial. Finally, the most highly socialized bees are termed eusocial and are characterized by cooperative brood care, division of labour and reproductively isolated castes (Lin and Michener, 1972). In these species, there is considerable reproductive skew as reproduction is mostly controlled by the queen. Although semisocial bees are similar to eusocial bees, the important difference is that the nests of the former consist of bees of one generation, while the nests of the latter consist of mothers and daughters (Michener, 1974).

Although many social bees fit into one of these social categories, there are others that may change their degree of sociality either over time or between colonies. These are thought as a primitively social group and therefore a very important group for the study of social evolution in bees (Sakagami, 1960; Lin and Michener, 1972).

In the halictine and allodapine bees, social relationships within colonies of one species may change with time or between colonies (Schwarz *et al.* 2007). In *Halictus sexcinctus*, some colonies are communal, with all females being reproductively

equivalent, while other colonies are eusocial having reproductive queens and workers with reduced fertility (Richards *et al.* 2004). These bees are proof that sociality has some degree of plasticity in some species.

Perhaps the most interesting are bees that may live solitarily or socially within the same population. These bees are termed “facultatively social” and provide a unique opportunity to study social evolution, since behaviours that may lead to social interaction can be observed accurately at the level of the individual. An example is the orchid bee, *Euglossa viridissima*, which forms both single and multiple female nests (Cocom Pech *et al.* 2008). By comparing these different nests, it was found that single female nests were parasitised more often than multiple female nests, which provides an evolutionary explanation for why bees live in social groups. The genus *Xylocopa* often displays within-population social variation and thus serves as an excellent model to study social evolution (Stark *et al.* 1990; Bernardino and Gaglianone, 2000; Hogendoorn and Velthuis, 1998). Facultatively social bees such as the above are valuable for the study of social evolution, since it is possible to compare the costs and benefits of both solitary and social living within the same population. This provides insight into how and why social groups have evolved.

Division of labour

A major step in the evolutionary transition from solitary to social behaviour is the division of labour (Keller and Reeve, 1994). This is a necessary component to eusocial nests, and it is expected to arise due to behavioural differences among solitary individuals (Page and Erber, 2002). When individuals of *Ceratina flavipes*, a mostly solitary bee, are

experimentally forced to nest together, one becomes an egg-layer and guard and the other acts as a forager (Sakagami and Maeta, 1987). This implies that specific social roles are intrinsic in individuals, but only become apparent once these individuals are in a group. For this reason, individuals can be thought of as specialists that have unique contributions to their group (Page and Erber, 2002). Sakagami and Maeta's (1987) experiment with *Ceratina flavipes* also illustrates that division of labour can be the first apparent shift towards sociality, and that the first roles to arise are egg-laying and foraging. Considering that division of labour is the most fundamental feature of advanced social bees (Michener, 1971), examining in detail the roles of foraging and/or egg-laying may give clues about steps involved in the evolution of castes and sociality.

The degree of division of labour may be capable of changing in some species. *Lasioglossum (Evylaeus) apristum* is known to have a life cycle with two distinct phases that consist of a solitary phase in spring and a eusocial phase in summer. In the eusocial phase, there is division of labour as evidenced by egg-layers and foragers (Miyanaga *et al.*, 1999). In a study with *Halictus sexcinctus*, it was found that there were both communal and eusocial nests in a single population (Richards *et al.* 2003). Socially polymorphic bees such as these, can be identified on the basis of division of labour, as bees in solitary or communal nests do all of their own foraging and egg-laying, while in eusocial nests, reproduction is skewed in favour of few individuals. For this reason, understanding which bees are doing the egg-laying and foraging is important in deducing the kind of sociality in a nest.

In advanced eusocial bees, such as the honeybee, the roles of egg-laying and foraging are completely divided (Michener, 1974). This means that the fitness of the offspring is determined by some interaction between the egg-layer (queen) and the foragers (workers). In bees that assume both the foraging and egg-laying roles, this fitness relationship can be more directly examined, as one female provides all the resources (egg, brood cell, food) which are required for the survival of her offspring. The fitness of a forager and her offspring can, thus, be measured by observing forager behaviour since foraging ability is directly related to both egg-laying and provisioning ability (Danforth, 1990; Kim 1997; Burkle and Irwin, 2009).

The degree to which division of labour appears in social groups may be predicted using reproductive skew models (Keller and Reeve, 1994). In solitary bees, reproductive output is directly related to work input. In other words, a bee that is more effective at foraging and provisioning will also have higher reproductive success (Figure 1a). In communal nests, two or more females nest together but raise their own offspring independently and therefore show no reproductive skew (Figure 1b). In nests of semisocial or eusocial bees, a few females (queens) have a high reproductive output to work input ratio, while most (workers) have a very low reproduction to work ratio. This model is considered to be high skew. Some workers can be reproductive in some cases, and are queen-like in the sense that they get much more reproductive output than the average worker (Richards *et al.* 2005; Figure 1c).

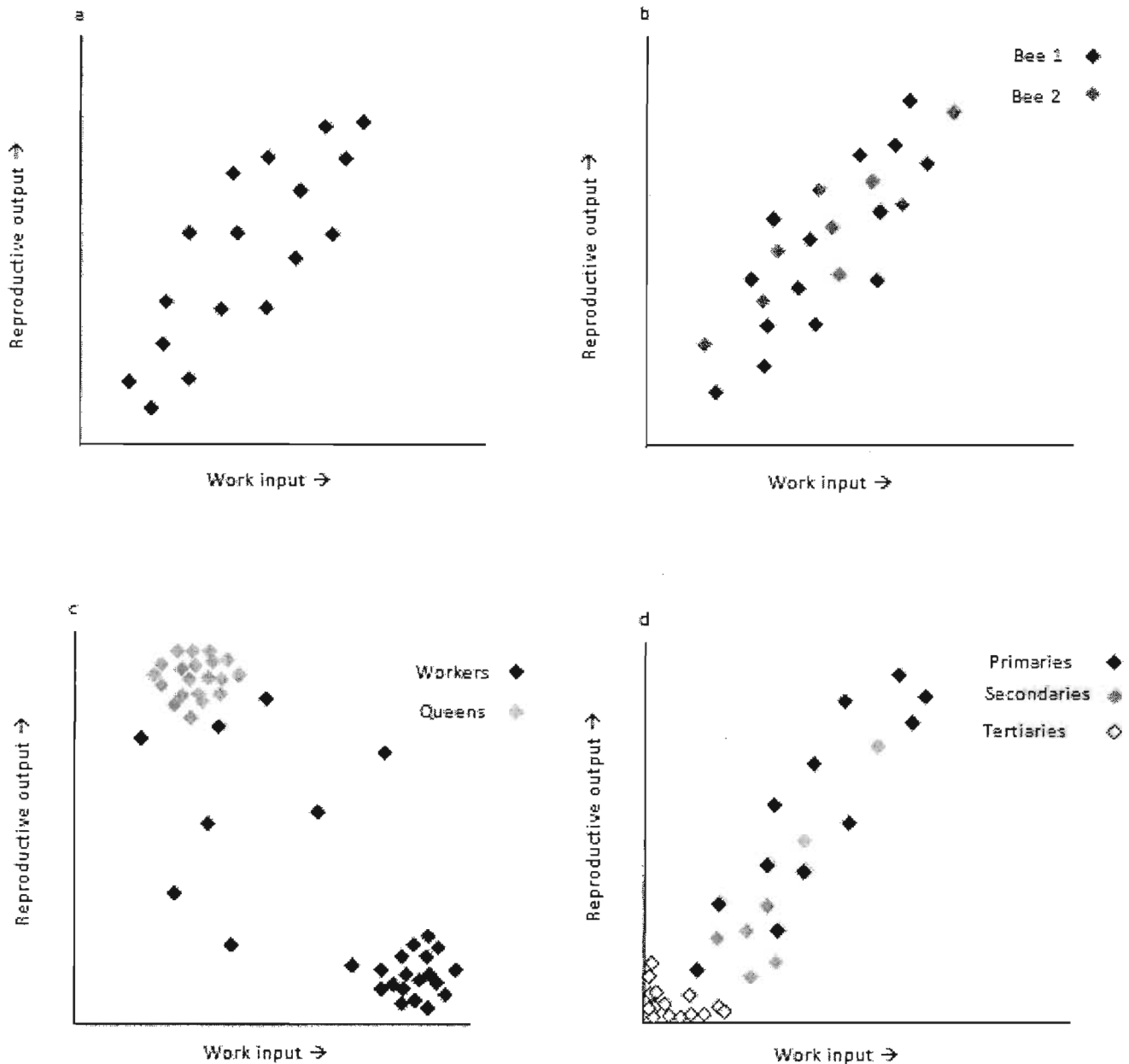


Figure 1: Division of labour in solitary (a), communal (b), and eusocial (c) bees, and in social *Xylocopa* (d). Solitary and communal bees have an approximately an equal amount of work input and reproductive output, as females are both the egg-layers and foragers. Eusocial nests show division of labour with queens doing little work and getting most of the reproductive output, while workers do most of the work and get little reproductive output. There are some queen-like workers that get more reproductive output than the average worker. Nests of *Xylocopa* show three different kinds of females. Primaries typically get the most reproductive output and do the most work, followed by secondaries which do less work and receive less reproductive output than primaries. Secondaries may replace dominants in a nest. Tertiaries typically do very little work and receive very little reproductive output. Note that in all cases and castes, there is individual variation of the work input and reproductive output ratio (Keller and Reeve, 1994; M. Richards, *in press*).

Ethology of foraging behaviour

The collection of resources is essential for an animal to survive. An individual's ability to forage efficiently is acted on by selection, such that animals that collect more or better resources have higher fitness than those that do not. Combined with the assumption that foraging behaviour is heritable, these are the fundamental components of optimal foraging theory, a concept for explaining the evolution of foraging behaviour (Pyke, 1984). Although this theory is favoured in the literature, it has been heavily criticised on the grounds that the link between forager behaviour and fitness needs to be fully understood before modelling can be used (Pyke, 1984; Pierce and Ollason, 1987; Raine et al. 2006).

Perhaps the most reliable methods of studying foraging behaviour are through studies among individuals of a population. Since selection acts at the level of the individual, and foraging behaviour shows plasticity among individuals of a population, studies that examine foraging strategies of individuals within populations in their natural environment give a clearer picture of the link between forager behaviour and fitness (Raine et al. 2006, Page and Erber, 2002; Pyke 1984).

The diversity of foraging activities can be understood in terms of response thresholds. An individual is “turned on” to forage when an external stimulus passes a particular threshold and “turned off” when the stimulus is below the threshold and elicits no response (Page and Erber, 2002). This has been demonstrated in colonies of *Apis mellifera* (honeybees) in which different classes of foragers (water, pollen, nectar) were each found to have a unique threshold concentration of sucrose solution that was required

to turn on their particular foraging behaviour (Pankiw and Page 2000). Other studies also demonstrate that perception of a stimulus in order to elicit a behavioural response can be specific to a particular group within a population of the same species (Endler *et al.* 2001; Chittka *et al.* 2003). These studies illustrate that individual behaviour is influenced by individual perception of certain stimuli and that these differences are inherent to individuals. These responses are not completely fixed, however, as it has been shown that learning in foragers changes the probability that an individual will respond to a specific threshold (Page and Erber, 2002).

Bee larvae are entirely dependent on the ability of adult female bees to collect and provide resources to them. Females collect both nectar and pollen from flowers, and either feed it directly to their larvae, or provide a provision mass on which an egg is laid and a developing larva feeds (Michener, 1974). In most bees, the mother seals the egg and provision mass into a compartment in the nest, referred to as a brood cell (Michener, 1974). The egg hatches, and the developing larvae feeds on the provisions. Once the provisions are consumed, the larvae enters the pupal stage, until finally emerging as an adult. It is important to note that the resources used by the offspring from the egg stage to the adult stage come entirely from the provisions that are provided by the mother.

The rate at which resources are brought back to the nest is termed the provisioning rate. There are a number of ways to quantify a female's provisioning rate, one of the most common being the number of foraging trips per day (Neff, 2008). Bees of the genus *Augochlorella* routinely make 1 to 2 trips a day, *Lasioglossum imitatum* females make 1 to 7 trips (Michener, 1974), while others such as *Xylocopa pubescens*

make 1-15 trips (Gerling *et al.* 1983). Provisioning rate is dependent on the amount of pollen or nectar that can be carried by an individual forager, the amount of time it takes to provision a cell and the amount of resources each cell requires, all of which vary strongly depending on the species (Neff, 2008).

Daily foraging patterns are species-specific. Some bees forage consistently throughout the day, reaching the highest intensity at noon (a unimodal pattern), while others reach the highest intensity before and after noon (a bimodal pattern; Wang *et al.* 2009). Total foraging time per day also depends on species. While many species of bees tend to forage for a 12 hour period (Richards, 2004; Wang, 2009), some are capable of foraging for up to 18 hours per day (Gerling *et al.* 1983).

Since bee larvae do not forage, there is significant selective pressure on the egg-laying, foraging and provisioning abilities of adult female bees, which contribute directly to an offspring's fitness. In a foraging study of *Calliopsis persimilis*, females that performed more pollen trips made larger provision masses, which resulted in larger offspring (Danforth, 1990). *Lasioglossum zephyrum* provisions masses of varying protein concentrations, and those with higher protein concentrations result in larger offspring (Roulston and Cane, 2002). Other studies have demonstrated that having more or higher quality resources results in larger offspring (Johnson, 1990) and/or faster larval growth rates (Bourke and Irwin, 2009). In this way, more efficient foragers make more fit offspring, which is often evident in the size of the offspring.

Large size in bees comes with many benefits. Larger bees are able to forage across greater distances (Greenleaf *et al.* 2007; Gathmann and Tschardtke, 2002) and for

longer durations (Gathmann and Tschamntke, 2002). They are also able to carry more pollen per trip and may have a higher provisioning rate (Neff, 2008). Bigger females are known to construct brood cells more quickly (Kim, 1997), and they also construct larger brood cells, which allows space for more provisions (Alcock, 1979). Larger females also lay bigger and more eggs, and are more likely to usurp smaller females in social nesting conditions (Kim, 1997).

Sociality in the genus *Xylocopa*

Bees of the genus *Xylocopa* nest in dead or decaying wood. The distribution of these substrates has a strong effect on the occurrence, distribution, and nesting habits of the bees themselves (Hurd, 1958; Michener, 1974). Nests dug in twigs consist of one linear tunnel, while those dug in larger substrates (tree branches, timber) have several branching tunnels (Steen and Schwarz, 2000). Bees nesting in larger substrates often form aggregations in which there are multiple nests in the same piece of wood, which is not possible in twig nests (Michener, 1974; Stark *et al.* 1990).

Most carpenter bees in the genus *Xylocopa* nest solitarily, but there are several species that show some degree of social nesting, and this occurs more often in species that nest in aggregations (Stark *et al.* 1990; Bernardino and Gaglianone, 2000; Hogendoorn and Velthuis, 1998). The degree or type of sociality varies greatly within and between species, and virtually every definition of sociality has been used to describe them (Bernardino and Gaglianone, 2008; Hogendoorn and Velthuis, 1998). Since many species of carpenter bee are facultatively social, they serve as models for studying the

evolutionary transition between solitary and social nesting as well as the early stages of social evolution (Steen and Schwarz 2000).

Since carpenter bees reuse nests, competition for nests often results, even though there are usually some unoccupied nests in an aggregation (Bernardino and Gaglianone, 2008; Gerling *et al.* 1989; Stark *et al.* 1990). Intraspecific competition for nest sites, proximity to food resources and defence against nest usurpers are likely reasons that some carpenter bees demonstrate social nesting (Steen and Schwarz, 2000).

In solitary carpenter bees the mother alone constructs brood cells, forages and provisions her young (Michener, 1974). In social carpenter bees the role of forager falls to the dominant female, who also constructs the brood cells and lays the eggs (Gerling *et al.* 1989). All female carpenter bees can be reproductively active, so in nests with two or more females, there is a high degree of reproductive competition (Steen and Schwarz 2000; Hogendoorn and Velthuis, 1998). Dominance is achieved by aggression, and fights between females are common. In species such as *X. sulcatipes*, the dominant consumes any eggs laid by the subordinate (Stark *et al.* 1990). The subordinate bee remains in the nest as a guard, preventing other conspecifics from usurping the nest. While the presence of guard bees helps to increase the reproductive output of the dominant, nest take-overs by the subordinate are common and can result in a reversal of dominance (Hogendoorn and Velthuis, 1993). In *X. pubescens*, guard bees have a 50% chance of gaining dominance during a struggle, which is higher than the probability of finding a nest elsewhere and gives the guard an incentive to stay within the nest (Hogendoorn and Velthuis, 1995).

Guarding by subordinate females is often explained using kin selection or hopeful reproductive theories (Hogendoorn and Velthuis, 1998; Steen and Schwarz, 2000). If the guard is related to the dominant, then helping to ensure the survival of the offspring increases the inclusive fitness of the guard. If the dominant dies or leaves, the guard may inherit the nest. In some bees like *X. pubescens*, it is argued that the dominant has 100% of the direct reproductive fitness (Hogendoorn and Velthuis, 1998), but other authors contend that this cannot be true since there is too little incentive for the guard to remain in the nest (Dunn and Richards, 2003). A guard would be much more likely to stay if in a nest usurpation event, all of her brood were not destroyed, so that she may stay and ensure the survival of some of her own offspring (Dunn and Richards, 2003). This has been observed in some species of *Xylocopa* where multiple females forage, have developed ovaries, and may produce offspring in the same nest (Camillo and Garofalo, 1989). In other species such as *X. sulcatipes*, the dominant female destroys some, but not all of the guard's brood, which gives the guard a staying incentive as she is allowed to keep some of her brood (Stark *et al.* 1990).

In nests of socially polymorphic bees (*Xylocopa*), individuals can be separated in terms of activity. In the genus *Xylocopa*, individuals act as solitary bees in the sense that they do all their own foraging and egg-laying. In this way, the amount of foraging is predictive of reproduction. It is known that within colonies, some females do a lot of work (dominants), while others do less (subordinates) and some do very little, if any (Richards, *in press*). Reproductive skew likely favours the dominant, although some degree of reproductive output seems to be achieved by subordinates, which may be able to become dominants (Richards, *in press*; Figure 1.1d).

Movement between nests

Since many species of *Xylocopa* nest in aggregations (Michener, 1974; Gerling *et al.* 1989), and there is a tendency in the genus to reuse nests (Hogendoorn and Velthuis, 1995), it is not surprising that there are many cases when bees move from nest to nest (Peso and Richards, 2010). These movements can occur for a number of reasons. After the daughters become adults, they may disperse to look for suitable nests of their own, or they may stay in the natal nest to help their mother (Camillo and Garofalo, 1989). In the event of a nest usurpation by another adult female, both the mother and daughter of the usurped nest may abandon it and attempt nest usurpation elsewhere. In *X. pubescens*, nest usurpations are common (Hogendoorn and Velthuis, 1995), and this is likely the reason that many females relocate.

Foraging activity in the genus Xylocopa

Regardless of living conditions in *Xylocopa*, the foraging females do all their own foraging and nesting activities (egg-laying) for their offspring, while the other females (in social nests) guard the nest (Gerling *et al.* 1989; Velthuis and Gerling, 1983; Camillo and Garofalo, 1989). Since every forager is also an egg-layer, both the fitness of the forager, and the forager's offspring may be measured by examining forager behaviour.

Detailed studies of the foraging activity of individual species of *Xylocopa* are rare, but those that do exist indicate that foraging activity is both irregular and variable. A foraging study of *X. pubescens* indicated that there are extended periods in which individuals ceased pollen collection and flight activity. Furthermore, the number of

foraging trips per day per female ranged from 1 to 15. These sporadic foraging patterns seem not to be caused primarily by environmental factors, but more likely by activities within the nest, such as brood cell preparation (Velthuis and Gerling, 1983). Another study concerning *X. ordinaria* revealed that provisioning time was very variable, both within and among individuals (Bernardino and Gaglianone, 2008). Within species variability is also evident in Gerling and Hurd (1983), that examined the number of bees flying during the day in *X. sulcatipes* and *X. pubescens*.

Sociality in Xylocopa virginica

Xylocopa virginica is a common species in eastern North America and can live up to two years (Gerling and Hermann, 1978). Within the nest, females construct brood cells and provision up to eight young. While females can nest solitarily, they often nest socially. In social nests, all of the foraging and egg-laying is done by the dominant female, while subordinate bees act as guards (Gerling *et al* 1989; Gerling and Hermann, 1978).

Within the nest, females appear to have a social hierarchy which consists of a dominant and two kinds of subordinates, secondaries and tertiaries (Richards, *in press*). These kinds of females were classified by the amount of work that females did during the breeding season, based on wing and mandibular wear at the end of the season. Dominants had the most wing wear, followed by secondaries and then tertiaries. These varying degrees of wing wear were suggestive of both foraging and brood provisioning activity. Dominants, theoretically, did the most foraging and provisioning, while

secondaries did less, and finally tertiaries did very little of these activities (Richards, *in press*).

Aggression tends to determine the dominance hierarchy of social bees of the genus *Xylocopa*, and factors such as age and size determine aggression (Hogendoorn and Velthuis, 1999). In *X. virginica* males, bigger males tend to guard better territories than smaller males, which guard peripheral territories (Barthell and Baird, 2004). In females, size variation does exist (Skandalis *et al.* 2009), although this has not been tested in the different classes of females within the nest. Since dominants are larger than subordinates in other *Xylocopa* (Hogendoorn and Velthuis, 1999), the dominant foragers should be older and/or larger than subordinates.

Peso and Richards (2010a) investigated whether or not nest-mates were capable of recognizing each other. By testing nest-mates and non-nest-mates within a circle tube, it was concluded that bees could recognize each other since a lower level of aggression was expressed between the former compared to the latter.

Size variation in X. virginica

Like other species of *Xylocopa* (Michener, 1974; Gerling *et al.* 1989), *X. virginica* nests are clumped in aggregations, and females move from nest to nest. In a study of the Brock University *X. virginica* population, it was found that half of females move from their home nests, while half stay in their home nests. Of the females that do relocate, some move from their home nests and then back again, and others repeatedly relocate throughout the season (Peso and Richards, 2010b). No definitive reason has been established as to why some females relocate to different nests. The tendency of bees to

relocate may reflect the lack of a staying incentive that a subordinate receives from the dominant of the nest. Since *X. virginica* can nest solitarily, which results in higher reproductive fitness than social nesting (Richards, *in press*), it is possible that subordinates may leave in order to try to increase their reproductive fitness by nesting alone. Due to the small number of solitary nests that occur in nature, however, moving is likely risky, and social nests may provide subordinates with a better chance at reproduction than they would experience if they left the nest (Richards, *in press*). For this issue to be addressed, it needs to be known how many females are successful in reproducing after moving.

Foraging and social behaviour of X. virginica

The foraging activity of *X. virginica* has not been studied in detail or with a large population. A study examining the foraging behaviour of *X. virginica* revealed that females tended to live in groups of two or three, with one bee per nest doing all the foraging and egg-laying (Gerling and Hermann, 1978). The non-foraging bees were mostly inactive for the season, flew rarely, and had little to no wing or mandibular wear. In contrast, foragers flew on most observation days and did anywhere from one to six foraging trips per day (Gerling and Hermann, 1978). Since *X. virginica* females do all of their own foraging and provisioning, the amount of foraging is predictive of how much brood they raise, which makes foraging behaviour a very useful measure of fitness.

Given that within a social nest of *X. virginica*, different kinds of females can be identified by examining the division of labour (Richards, *in press*), studying individual foraging behaviour may be used to describe more appropriately how social nests function.

Objectives and Hypotheses

The overall goal of this study was to investigate whether foraging behaviour can be used to identify the social roles of females living in social nests of *X. virginica*. To do this, I have split my thesis into two main sections, the first deals with individual variation in foraging strategies, and the second looks at the forager roles within the nests.

In the first section, my objective was to determine clearly the range of individual forager variation in *X. virginica*. Since foraging studies have shown a large degree of variation in other foragers of the *Xylocopa* genus (Gerling and Hurd, 1983), it was hypothesized that there would be a large degree of variation between foragers of *X. virginica*. Since foraging effort is measured primarily by the number of foraging trips and daily provisioning time (flight time, handling time), different foraging strategies could be reflected in differences of either or both of these variables. Given that both of these measures vary widely among individual foragers of *X. pubescens* (Velthuis and Gerling, 1983), it was hypothesized that *X. virginica* foragers would also exhibit variation in the number of foraging trips and provisioning time. It was investigated whether different aspects of foraging behaviour were associated with body size. It was predicted that larger bees would collect more pollen than smaller ones, as evidenced by increased foraging time, as well as more foraging trips.

Social roles might be indicated not only by foraging patterns, but by whether or not females are even engaged in foraging behaviour. Therefore, I compared foragers to non-foragers. It was hypothesized that there would be more non-foragers than foragers since this was the case in previous studies with *X. virginica* (Gerling and Hermann,

1978). Since foraging is a dominant behaviour and body size is related to dominance (Hogendoorn and Velthuis, 1999), foragers were predicted to be larger than non-foragers. Foragers were also predicted to fly more often than non-foragers as the latter would not be collecting pollen at any point in the season. The notion that non-foragers might be adopting a waiting strategy was evaluated. Since females can live for two years and only have a limited amount of foraging energy, it is possible that some subordinate females forego current breeding opportunities and save their energy until the next year, giving them a greater chance of taking over the nest as the dominant.

The major objective of the second section of this study was to use foraging behaviour as an indicator of division of labour within nests of *X. virginica*. Since *X. virginica* is facultatively social, populations of this species serve as an excellent model for studying social effects on foraging behaviour. The social nests of *X. virginica* are arranged in a dominance hierarchy. Theoretically, the dominant bee forages the most and gets the most reproductive benefit, while the subordinates must get less (if any) reproductive output, and should also do less foraging than the dominant.

The major objective, that foraging behaviour can be used to separate females into the categories of dominant and subordinate, was investigated by using two different models to categorize the dominance hierarchy. The first model, referred to as the “total resources model,” separated females within each nest according to the total observed number of pollen trips that females performed. This produced two kinds of foragers for each nest: the dominant female, which had the highest number of observed pollen trips and, therefore, had the highest reproductive success, and the subordinate females, which

collected less pollen than the dominant and, therefore had lower reproductive success. The second model was based on forager sequence and is referred to as the “forager sequence model.” In this model, the dominant bee was designated as the first bee of the nest to make a pollen trip (also referred to as the “first forager”), while the subordinates or “subsequent foragers” of the nest were the remaining bees. In both models, subordinate bees that did not forage were referred to as non-foragers, which were bees that never did a single pollen trip.

In order to investigate the social implications of foraging behaviour, it was necessary to classify nests as solitary or social. In addition to this, it was necessary to know the proportions of foragers and non-foragers in nests. Once this was known, it was possible to investigate foraging behaviour both between and within nests. Within nest comparisons were made between foragers in order to see how the social organization of nests is revealed in terms of foraging behaviour. It was hypothesized that dominant bees should be larger and forage more (as evident by number of pollen trips) than subordinates, who might or might not forage at all. Another objective was to investigate the timing of foraging within a nest. In nests with multiple foragers, it was hypothesized that the first bees to forage should be larger and better foragers than subsequent foragers.

Another objective of this thesis was to investigate the consequences of relocating to new nests, in order to address whether females that relocate can raise as many brood as bees that do not. Nest relocation is common in bees of the genus *Xylocopa* (Camillo and Garofalo, 1989; Hogendoorn and Velthuis, 1995; Peso and Richards 2010b), but it is still not known what implications relocating nests has for foraging bees. Bees that never

relocated nests (home bees) and bees that relocated at least once (relocators) were compared in terms of many factors that may contribute to reproductive success (body size, foraging behaviour, foraging season length) in order to address this issue. It was hypothesized that home bees should be larger and better foragers than relocators, which likely use up their energy finding nests and do not collect as many resources for their young as home bees.

Finally, wing wear in *X. virginica* was also investigated. Measures of wing wear (accumulation and rate) were introduced and used to compare the different kinds of females as mentioned above, in order to see if certain groups of females exhibit more wing wear than others. Since wing wear is positively related to mortality, and increased flight activity increases wing wear (Cartar, 1992), wing wear is an important indicator of foraging behaviour and nest movement as well and may be an important factor in comparing various female foraging and social strategies. I hypothesized that dominant bees would have higher wing wear than subordinates and that relocators should have more wing wear than home bees.

Methods

Study area

All observations were completed in Jubilee Court, at Brock University, St. Catharines, Ontario, Canada (Latitude, Longitude = 43.20, -79.17). Within the courtyard were 7 cedar benches where *X. virginica* have nested. Some benches housed as many as 30 nests, although not all were active in 2009. Surrounding the benches are 20 red maples and two large grass lawns (Figure 2).

A pilot study to establish observational techniques was carried out in 2008. Observations reported in this Thesis started 30 April 2009, after the first observed female pollen trip of the season and continued until 9 July 2009. The weather was quite rainy during the spring and summer of 2009 and observations were not done in the rain, as the bees do not forage in these conditions (Figure 3).

Bee capture and care

Bee capture and marking commenced after the first bee flight of the 2009 season was observed on April 24. ‘Peso traps’¹ were placed over all the nest entrances before daily flights began (Figure 4). Bee marking concluded after several days of catching yielded only already marked bees. Once a bee was caught, a replacement trap was added to the nest, and the trap containing the caught bee was chilled in a cooler for 10 minutes. After cooling, the bee was marked with a unique combination of two colours of enamel

¹ Bee trap named after Marianne Peso, who created and used the traps to capture *Xylocopa virginica* for her MSc research. Trap consists of a plastic drinking cup with a 1-2 cm hole cut out of the base and Parafilm™ applied over the open end. Trap is attached to the nest entrance via velcro strips.

paint on its thorax. Bees were also measured, and their wing wear scored, before being returned to their nests.

Body size and wing wear

Head width and intertegular (IT) width were measured in millimetres using electronic callipers when bees were first caught (Figure 5). Wing wear was scored at each capture. Wing wear score was based on a scale of 0 to 7, where 0 indicates an unworn wing margin, 5 indicates a completely worn wing margin, 6 indicates a reduction in wing length by wear on one wing and 7 indicates a reduction in wing length by wear on both wings (Figure 6).

There were 25 females for which at least two wing wear scores were obtained during the 2009 season. For these individuals both a wing wear accumulation score over the season (last observed wing wear score – first observed wing wear score) and a rate of wing wear per day $[(\text{last observed wing wear score} - \text{first observed wing wear score}) / (\text{last observed flying date} - \text{first observed flying date})]$ were calculated. In the analyses conducted involving wing wear, only these 25 individuals were used.

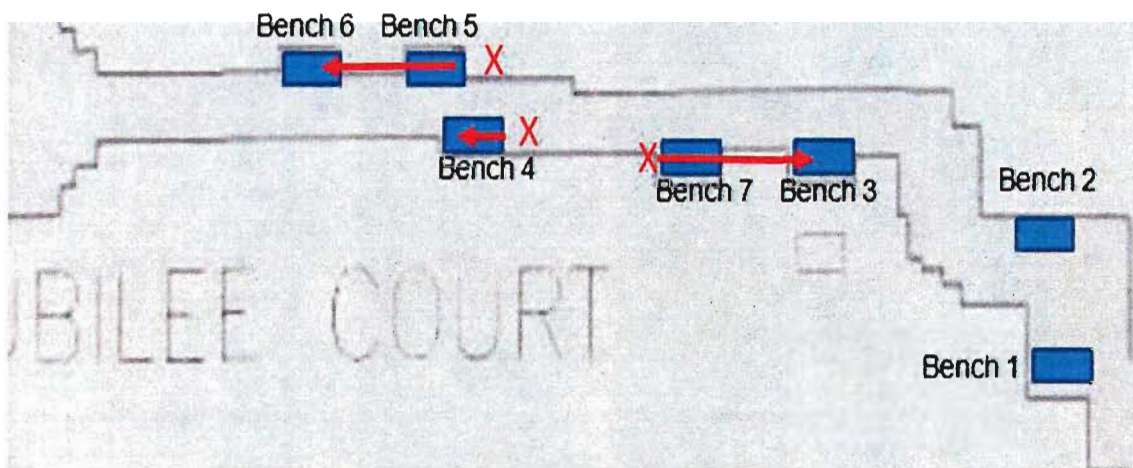


Figure 2: Bird's eye view of the area of study in Jubilee Court, Brock University (indicated in red; top). Picture obtained from Google Earth™. Diagram depicting the observation position (X), and direction (→) for the three observation days at the study site (bottom). Benches 5 and 6 had 16 active nests, Benches 7 and 3 had 12 active nests and Bench 4 had 14 active nests.

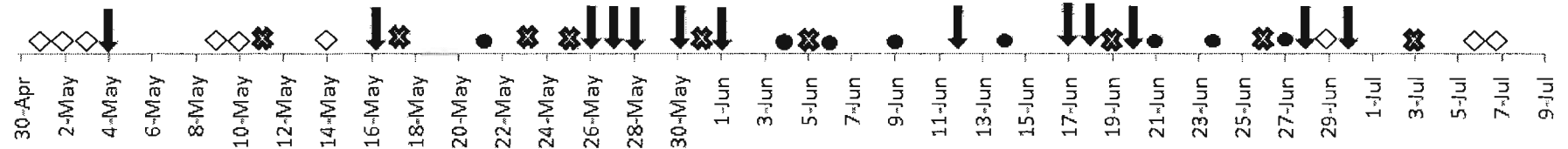


Figure 3: Full daily schedule of observations for the 2009 foraging season. Blank days indicate when bees were flying. Days in which it rained (↓), nest observations were done with camera (x), no observations were done (•), and when bees were not flying (◊) are all indicated.



Figure 4: Benches with 'Peso traps' placed over *X. virginica* nest entrances.

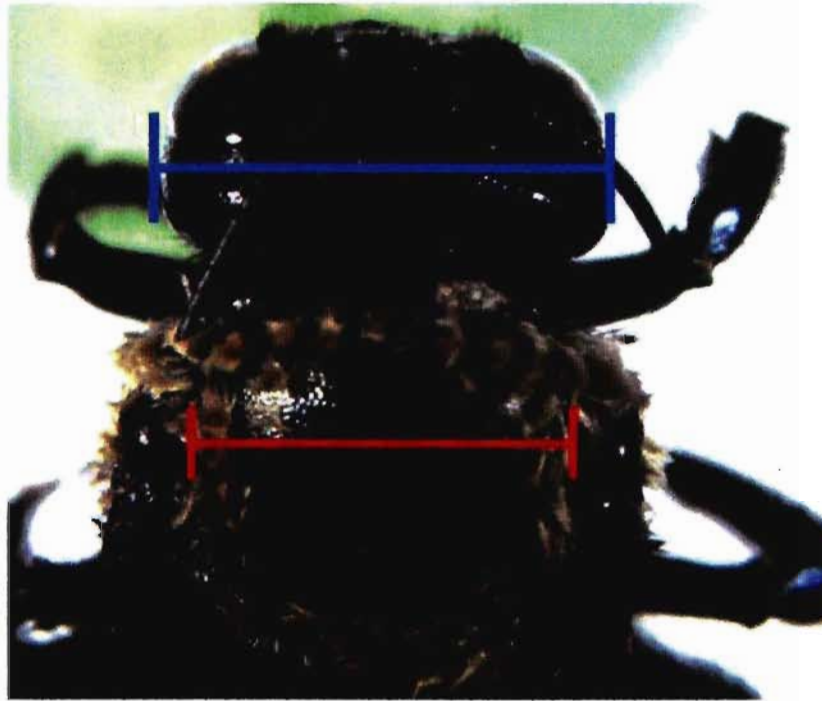


Figure 5: Measurements of head width (blue) and intertegular width (IT, red) of the female being measured. Measurements were done using electric callipers and recorded in mm. Figure obtained from Peso (2008).

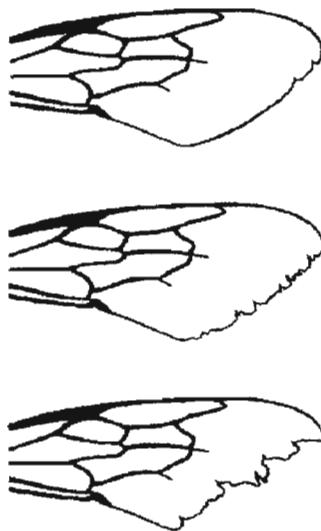


Figure 6: Diagram showing representative wing wear scores. The wing depicts a wing wear score of 1, the middle a 5, and the bottom is a 6. Figure obtained from Mueller (1993).

Pollen trip observations

To produce a complete foraging record for this nesting aggregation of *X. virginica*, the behaviour of all females was observed almost daily during the 2009 flight season. Observations began before the first pollen trip of the day, which was determined from the pilot study in 2008 to be about one hour after sunlight first touched the benches. Once foraging began, records were taken in a notebook of departure times (time leaving the nest), arrival times (time returning to the nest) and whether or not a female was carrying pollen when she returned. Observations were concluded for the day after there were no pollen trips for 2 hours. In the event of rainy, cloudy, or cold weather (below 15°C), observations were stopped, since *X. virginica* did not forage in these conditions. In the event of cold weather, benches were watched for 2 hours before being considered inactive.

Daily observations focussed on three sites: benches 3 and 4, benches 5 and 6, and bench 7 (Figure 2). Benches 3 and 6 had only one active nest each, so it was possible to watch these in addition to the other bench of the day. Benches 1 and 2 were omitted due to lack of activity as determined in the pilot study. Each site had an approximately equal number of active nests. Benches were watched along their length in order to see females enter and exit on both sides. An adjustable lab chair was used, so that it was possible to watch the bench from a higher vantage point. In the 2009 season, there were 32 observation days devoted to foraging activity.

Since observation time for this study was divided among three different sites and only one site was observed per day, individual bees were watched for 1/3 of their total

foraging season. Potentially, this means that 2/3 of the foraging done by any individual bee was not observed. A corrected estimate of total pollen collection was calculated by multiplying the average observed total pollen trips per bee per season by three, which would account for the time that the bees could not be observed.

There are several ways in which foraging effort can be measured. Pollen weight in total (or per brood cell) is the most exact measure of effort (Neff, 2008), but requires the observer to interfere with the nest and the bees, as the pollen loads need to be measured. Another way of estimating effort is to measure the number of pollen trips (Figure 6) per day as well as the duration of each trip (Gerling *et al.* 1983; Richards, 2004). These methods are appropriate since the number and length of pollen trips are both correlated with the mass of pollen brought to the nest (Danforth, 1990) and advantageous since the bees and nest remain undisturbed. A pollen trip describes the behaviour of a female leaving her nest, to return later with a visible pollen load which is carried on her legs.

Foraging effort of each female was measured by examining the number of pollen trips per day, the number of non-pollen trips per day, flight time per trip, handling time per trip, and provisioning time per trip. A trip that resulted in no visible pollen being carried is termed a non-pollen trip. The flight, handling, and provisioning times are all temporal components of a pollen trip (Figure 7). Flight time is defined as the time elapsed from a female's departure until her return to the nest. Handling time is the time spent inside the nest after a pollen arrival, until the next departure. Provisioning time is the sum of flight time and handling time. Note that handling time only occurs when a bee

is still foraging. From these measurements, total flying, handling and provisioning time per day per bee can be obtained by multiplying the number of trips per day by the mean of the desired time (Richards, 2004). In this way, foraging efficiency can be examined in more detail since it is known how females are spending most of their time.

A “bee-day” is an observational measurement of the amount of foraging (as measured in number of pollen trips, flight time, provision time etc.) done by an individual bee in one day. I use this term for analyses in which repeated observations of one individual foraging on different days are treated as separate and independent data points. This method is useful since it describes what an average foraging day is like for an average female on any given day.

Methods with video-probe

In addition to pollen trip observations, within nest observations were made using an Everest VIT Inc. VP300TM video-probe over the course of the summer. The video-probe was inserted into all nests, and all possible tunnels were explored. The number of tunnels containing brood cells was recorded, as were the sex and colour identification of any bees inside the nest at the time of observation. Due to the sharp corners of certain tunnels, not all could be accurately observed. Camera observations were made for a total of 9 days (May 11, 17, 23, 25, 31, June 5, 19, 26, and July 3, 2009) and were done mostly on days of low flight activity (after a rain, little sunlight), so as to maximize the number of bees inside the nest. Of the 42 nests with active foragers, 6 could not be entered by the video-probe, and 7 nests contained some tunnels that could not be entered.

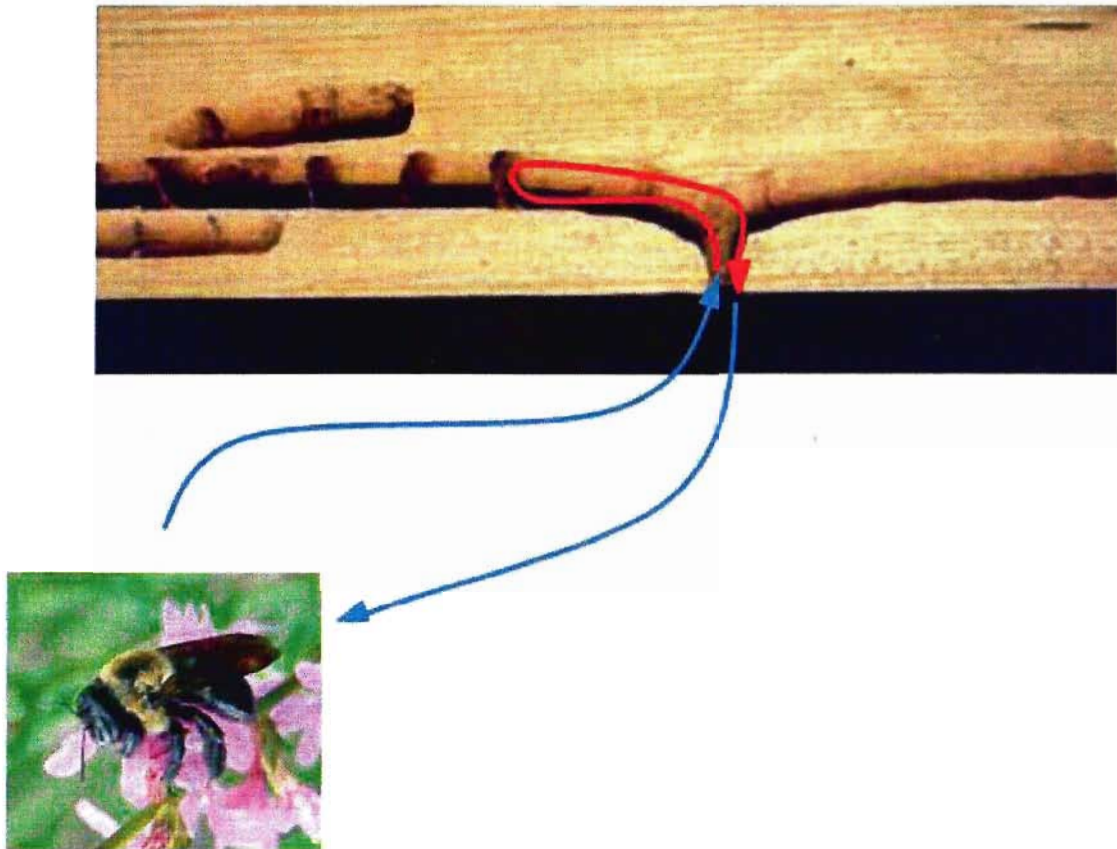


Figure 7: Figure depicting the temporal components of a pollen trip, with the nest shown as a cross-section. The blue arrows represent flight time, while the red arrows represent handling time. Provisioning time is the addition of flight and handling times.

The number of bees per nest was calculated by adding the number of foragers and non-foragers that were observed flying out of their nests. In the event that bees were inside (and rarely or never were observed outside) these were added to the total above, if it was confirmed that they were different bees. This could be confirmed when bees were observed in the nest, while the known nest inhabitants were known to be flying.

Nest relocation

Females that were known to have moved to another nest at least once were termed ‘relocators,’ and females that never relocated were termed ‘home bees’. The relocators and home bees were compared in terms of size (IT width and head width), flight season length (last date observed flying - first date observed flying), pollen foraging season length (last date observed foraging for pollen - first date observed foraging for pollen), general foraging behaviour (number of trips, number of pollen trips, flight time, handling time, etc.), and wing wear rate.

Dominance hierarchy

In the forager sequence and total resources models, the terms “dominant” and “subordinate” are used as operational definitions that are dependent on the model being addressed. The forager sequence model defines dominant bees as those that forage first, while the subordinates forage after the dominant. The total resource model defines dominants as the bees that were observed doing the most total pollen trips, while the subordinates did less than the dominant of each nest.

Statistical methods

All statistics were analyzed using SAS version 9.2. Variables were assessed for normality using the Kolmogorov-Smirnov test. For comparison with non-normal data, the ANOVA procedure was run on the ranked data. For tests involving quantitative variables, a linear regression was run was used on the ranked data. For normal variables, the above tests were run for the same underlying conditions, with the exception that the raw data were used. In cases where ANOVA was used with more than three categories, statistical differences among groups were identified using Tukey's post-hoc test. When an X-Y relationship was curvilinear, data were log-transformed (\log_{10}) and then a linear regression (GLM procedure) was run. Statistical tests were considered significant when $p < 0.05$.

A number of variables concerning foraging behaviour, body size, wing wear and important foraging dates were compared for first forager in each nest, subsequent foragers in a nest, and non-foragers, as well as dominant, subordinate and non-foragers. The variables compared were day of first observed flight, day of last observed flight, day of first observed pollen-collection, day of last observed pollen-collection, total number of days observed flying, total number of days observed pollen-collecting, total number of observed pollen trips, total number of observed foraging trips (pollen + non-pollen), mean flight time per trip, mean handling time per trip, mean provisioning time per trip, total flight time, total handling time, total provisioning time, head width, IT width, wing wear accumulation (score of wing wear incurred over season), wing wear rate (score per

day), and number of nests visited. The normality of these variables are indicated in Table 1 in the Appendix.

Results

Section 1 – Individual forager variation in *X. virginica*

Seasonal and daily foraging patterns in the X. virginica population

A total of 75 females were marked and observed for a total of 31 days in the 2009 season. There were two groups of bees, those that were observed foraging (carrying pollen) at least once (N=58), and those that were never observed foraging, but flew at least once (N=17). Pollen collection started 30 April and continued until early July. Females started flying in late April and continued into early July (Figure 8).

Non-foragers started flying in early May and mostly stopped by late June, although a few flights were seen in early July. The maximum number of non-foragers flying daily per site was 3, compared to 17 for foragers (Figure 8). Non-foragers were never seen on more than 5 days, while some foragers were seen on as many as 20 days. Foragers flew for more days than non-foragers (ANOVA, $F=24.5_{(1,74)}$, $p<0.0001$; Figure 9).

In terms of daily patterns, *X. virginica* foraged as early as 7:00AM through to about 7:00PM (Figure 10). During the morning few pollen trips were made, but as the temperature increased so did the number of pollen trips. The majority of pollen trips occurred by about noon, generating a unimodal pollen collection pattern (Figure 10). The distribution of the daily flights of foragers was similar to that of the total pollen trips, since the number of flying bees increased in the morning hours, peaked at around noon and then decreased slowly into the evening (Figure 10). Non-foragers started flying later

in the day and stopped flying earlier than foragers. The earliest non-forager flight was at 8:00AM and the latest was at 6:30PM. The highest number of non-forager flights were observed at 12:00PM.

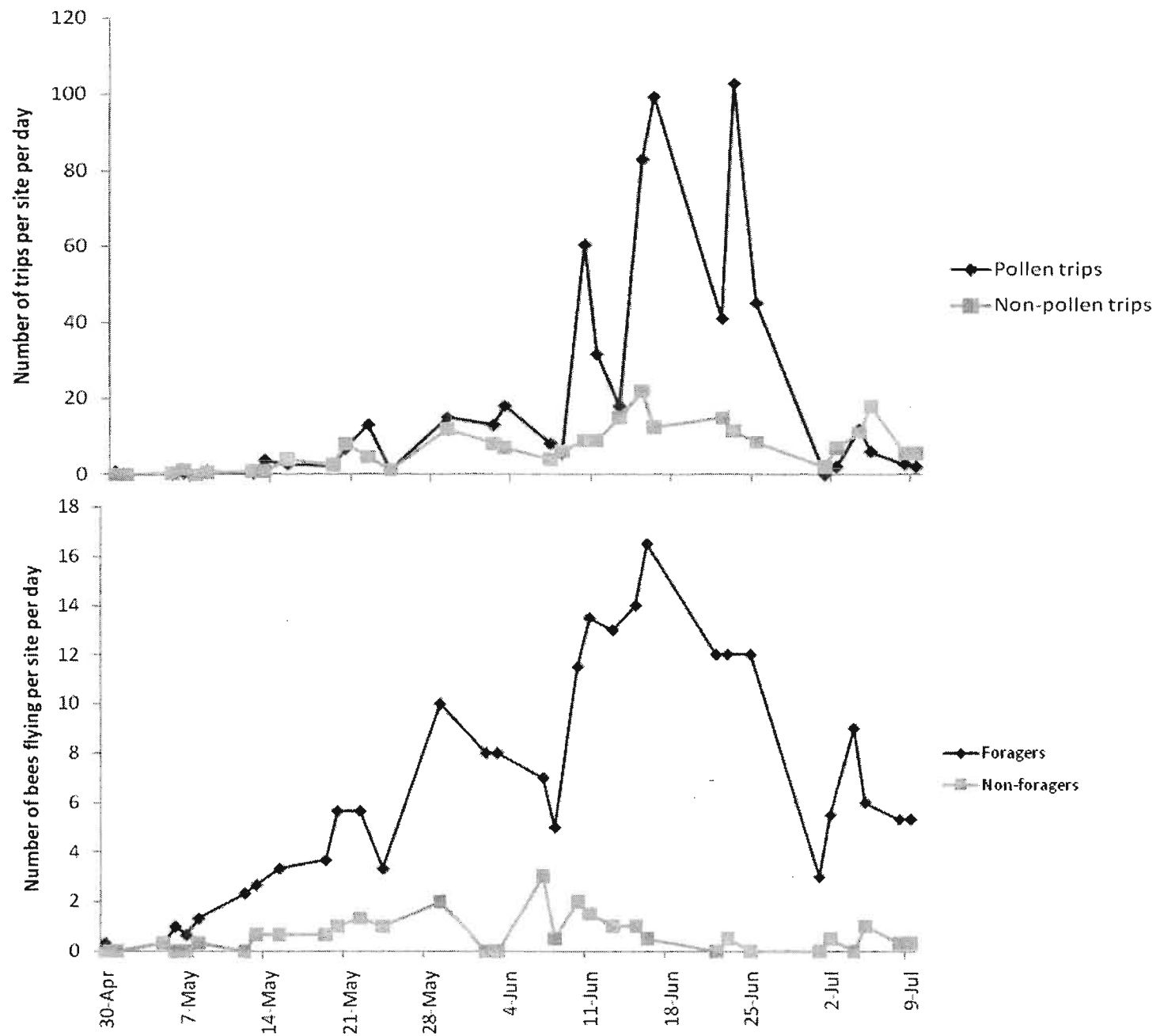


Figure 8: Seasonal pattern of pollen provisioning activity (top) and seasonal pattern of flight activity based (bottom) on daily numbers of pollen trips per site in the 2009 foraging season. There were 32 days of observation and 58 foragers and 17 non-foragers. A forager is a bee that brought pollen back to a nest at least once.

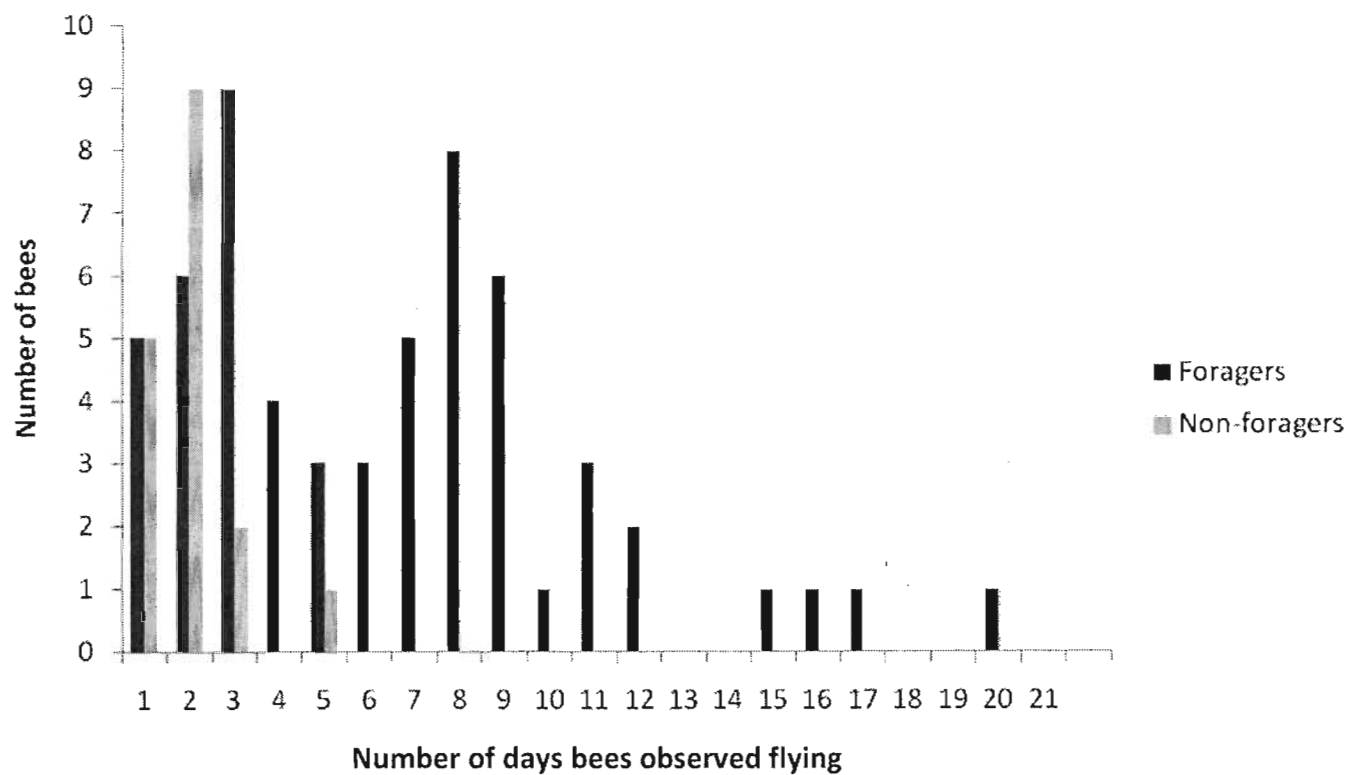


Figure 9: Total number of days observed flying for all females (N=75) in the 2009 season. There were 58 foragers and 17 non-foragers.

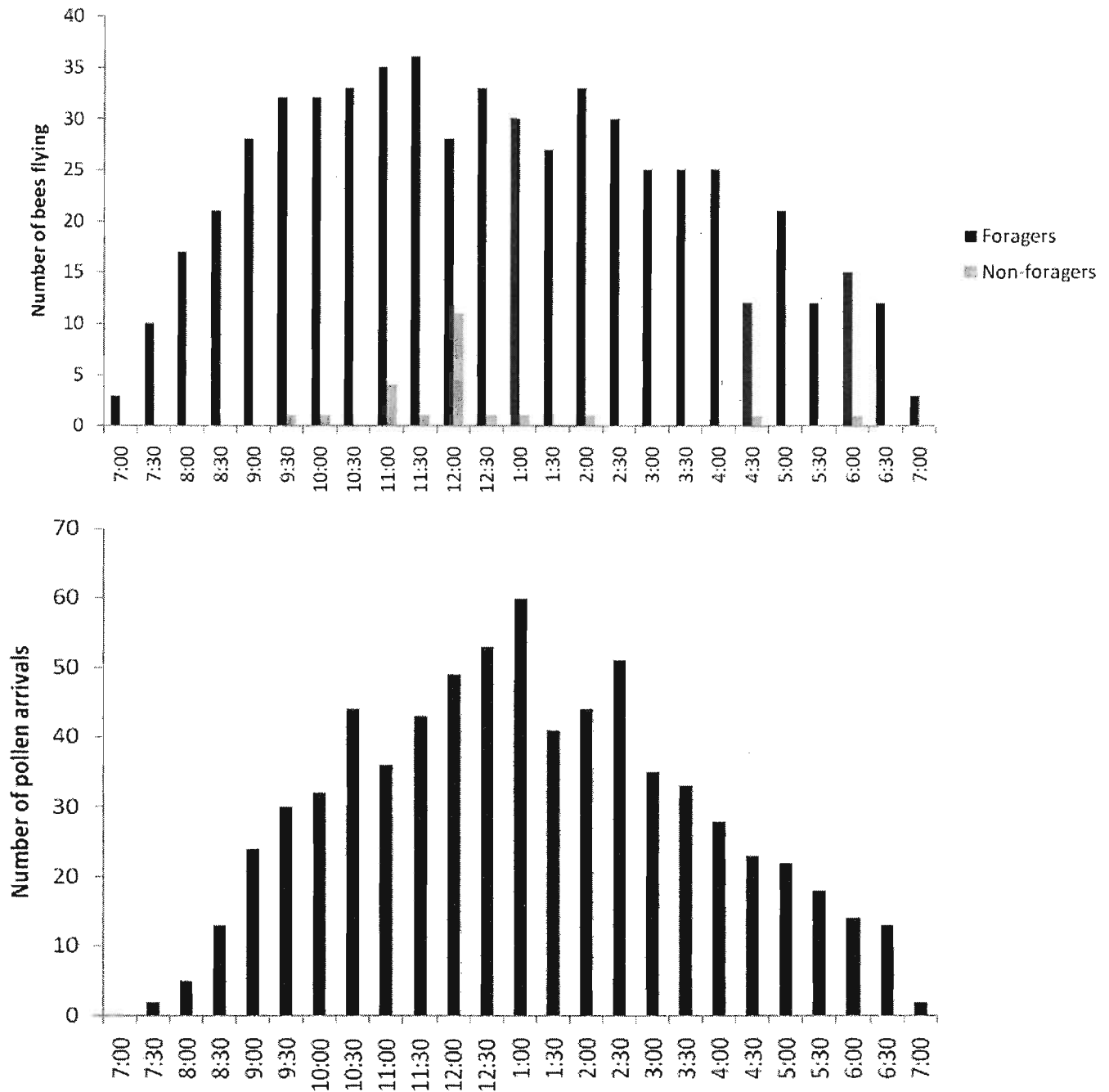


Figure 10: Daily pattern of flight activity of foragers and non-foragers (top) and daily pattern of pollen collecting activity (bottom) of foragers from all nests based on half-hourly number of bees flying for all 31 days observed in the 2009 foraging season. The distribution displays on an half-hourly basis when, and how many females were flying (top) and how many pollen trips were performed (bottom).

Individual variation among foragers

Within the forager group there were large differences in foraging patterns. The seasonal duration of flight activities ranged from only 1 day to more than 5 weeks (Figure 11). At the beginning of the season in early May, new females started foraging at a rate of 3-5 individuals per observation day. On 10 and 16 June, 8 and 7 new females, respectively, started pollen collecting, the highest numbers of new females to start foraging in the season. The highest frequency of total observed pollen trips occurred at June 16 and 23 (Figure 8). The high activity of June 16 was not due more trips per bee, but because more females starting collecting pollen at this time (Figure 11). On June 23 however, the increase in total pollen trips was due to individual females performing more pollen trips per day (Figure 8). This increased effort on June 23 of females was also the last peak of both flying and pollen collecting activity, after which both started to decline and they finally stopped in early July. On 25 June, 15 foragers stopped collecting pollen (Figure 11).

Foragers seemed to show differences not only in terms of when they started and stopped foraging (season length) but also in terms of intensity. Those that started to forage early in the season, also foraged fairly consistently across a long period of time (Figure 12A). Others concentrated their foraging effort into a shorter foraging season (Figure 12B), with some concentrating most of their foraging within a few days (Figure 12C). Some females started early in the season and/or stopped shortly after starting (Figure 12B). The earlier in the season that a female started foraging, the more pollen

trips she did compared to females that started foraging later in the season (linear regression, $F_{(1,57)}=9.06$, $R^2=0.1038$, $p=0.003$; Figure 13).

The observed number of pollen trips carried out by individual foragers over the entire season ranged from 1 to 65, with over half of foragers making fewer than 10 pollen trips (Figure 14). The total numbers of daily pollen trips per female were also variable and ranged from 0 to 19, with many days in which bees flew but collected no pollen (Figure 15). Not surprisingly, daily flight time, handling time and provisioning time were also very variable among foragers (Appendix Figures 1-3). The more pollen trips that were made in a day, the less time per trip was spent on flight and handling time respectively (linear regression, flight time: $F_{(1,188)}=20.1$, $R^2=0.10$, $p<0.001$; Figure 16; linear regression, handling time: $F_{(1,169)}=27.6$, $R^2=0.14$, $p<0.001$; Figure 16).

Of the 75 females marked, seven (6 foragers and 1 non-forager) were second-year bees that had overwintered twice. This was known because the old females still had markings from 2008, and they also had somewhat worn wings. In 2009, the first pollen trip recorded by a female in the season was from a second-year female on 30 April, 8 days before the next female started foraging.

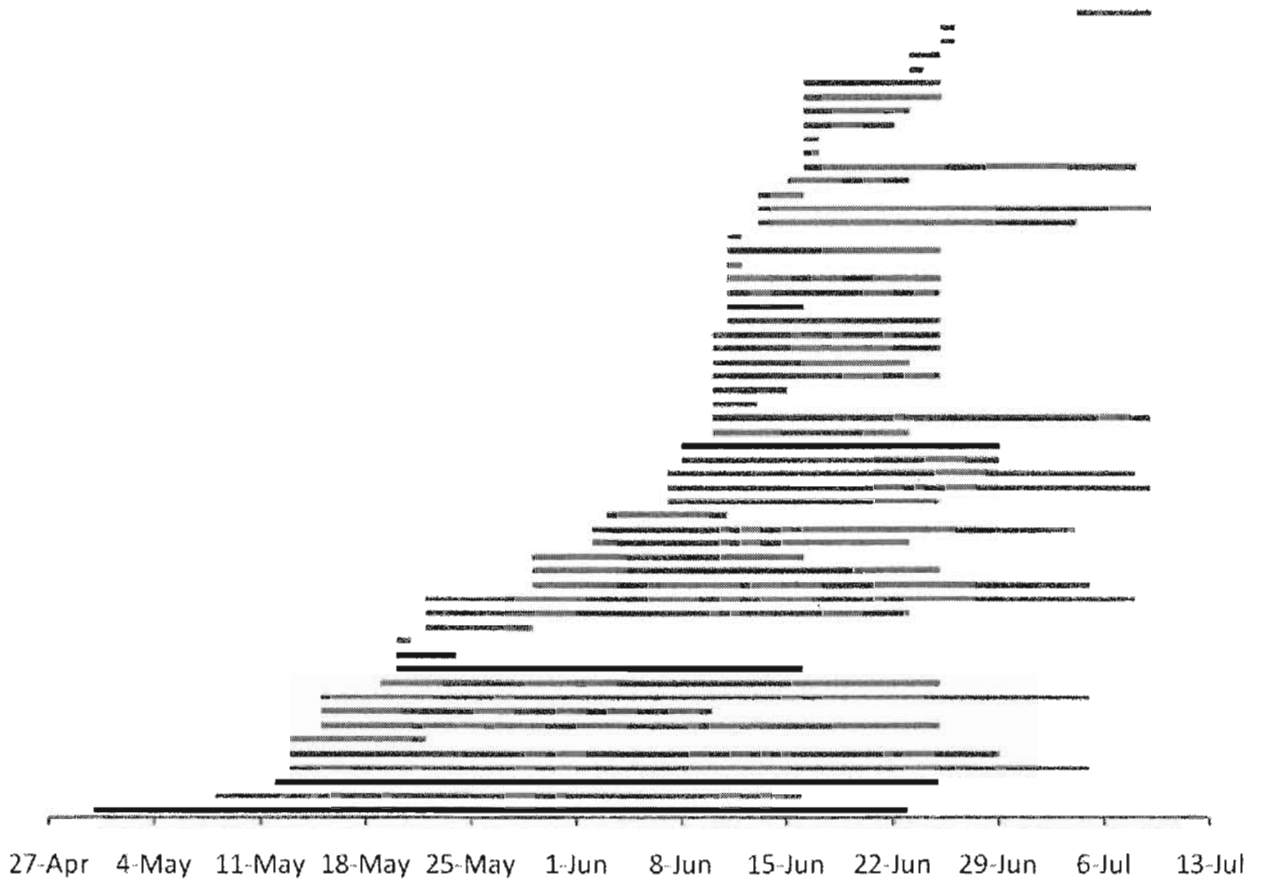


Figure 11: Individual foraging schedules depicting the first (left edge of the bar) and last pollen trip (right edge of the bar) for all females that were observed to forage at least once in the 2009 season (N=58). Gray colour is used to distinguish first-year females, while black is used for second-year females. Each bar represents a different female.

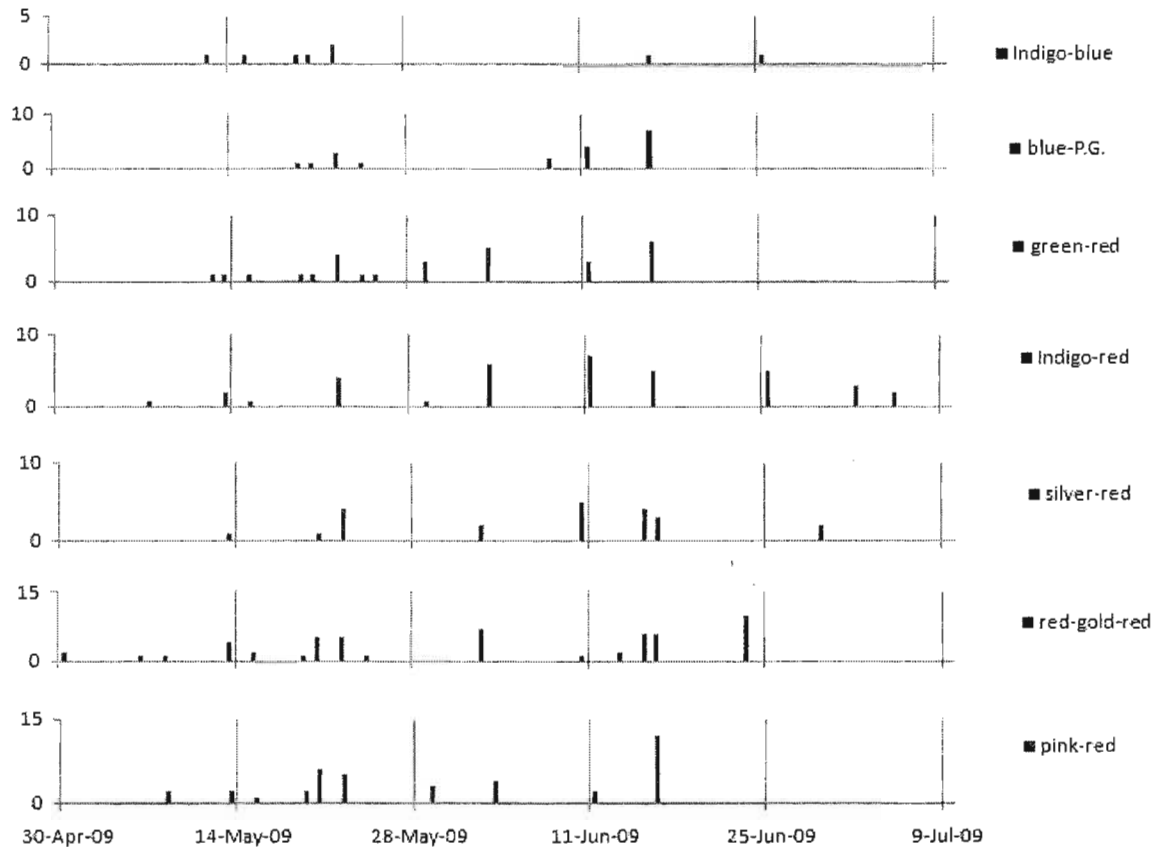


Figure 12a: Pollen foraging schedule of females that did at least two days of pollen collection (N=36) grouped based on foraging period of duration (start foraging date to end foraging date) and foraging intensity. Group A females (N=7) started foraging early (early to mid May) and had a long foraging season (duration of ≥ 27 days) when compared to the other foraging groups (Figure 15B; Figure 15C). Group A tended to forage more consistently when compared visually to the other groups.

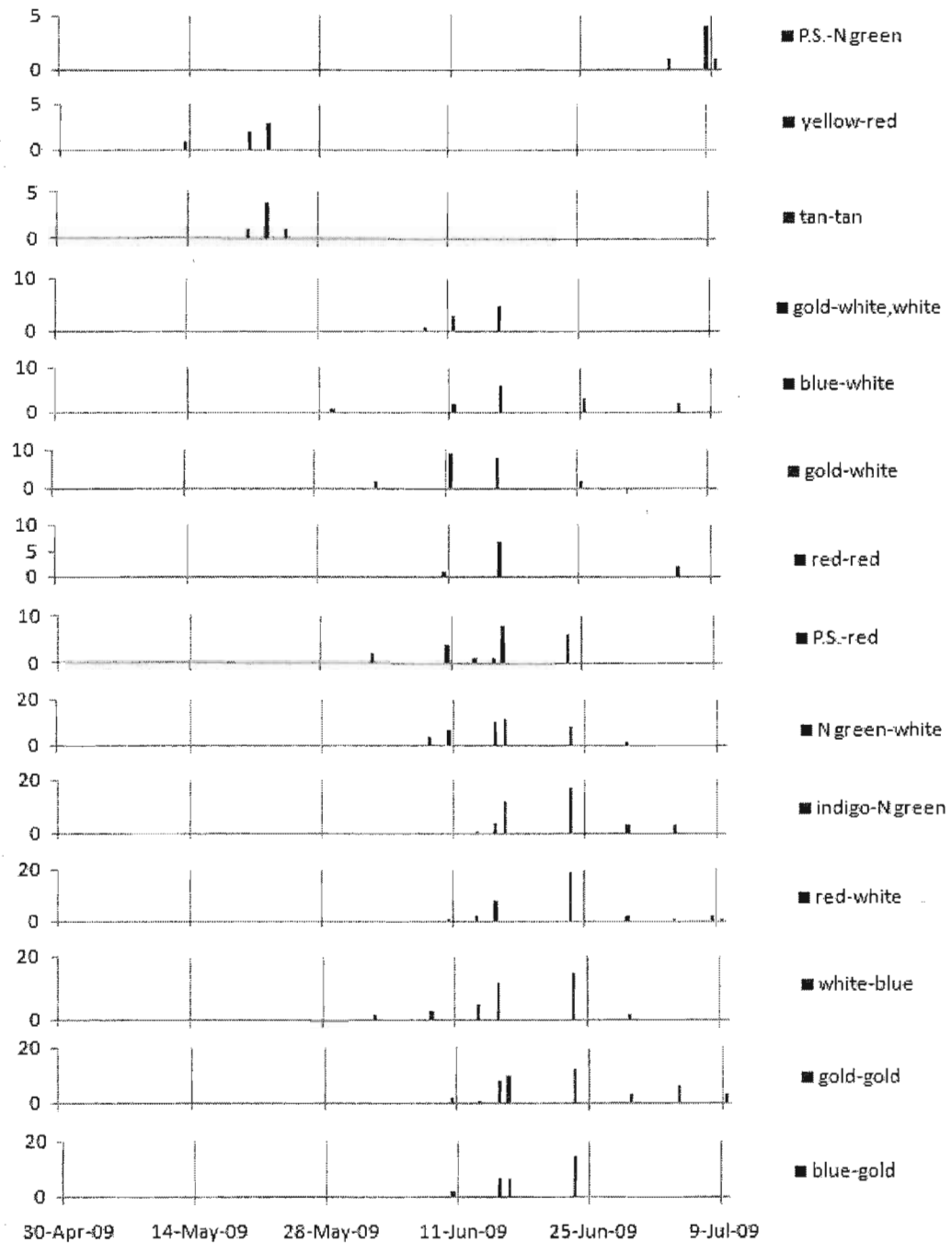


Figure 12b: Pollen foraging schedule of group B females (N=14). Group B females had a shorter foraging season and/or started later in the season (late May – early July) than group A females.

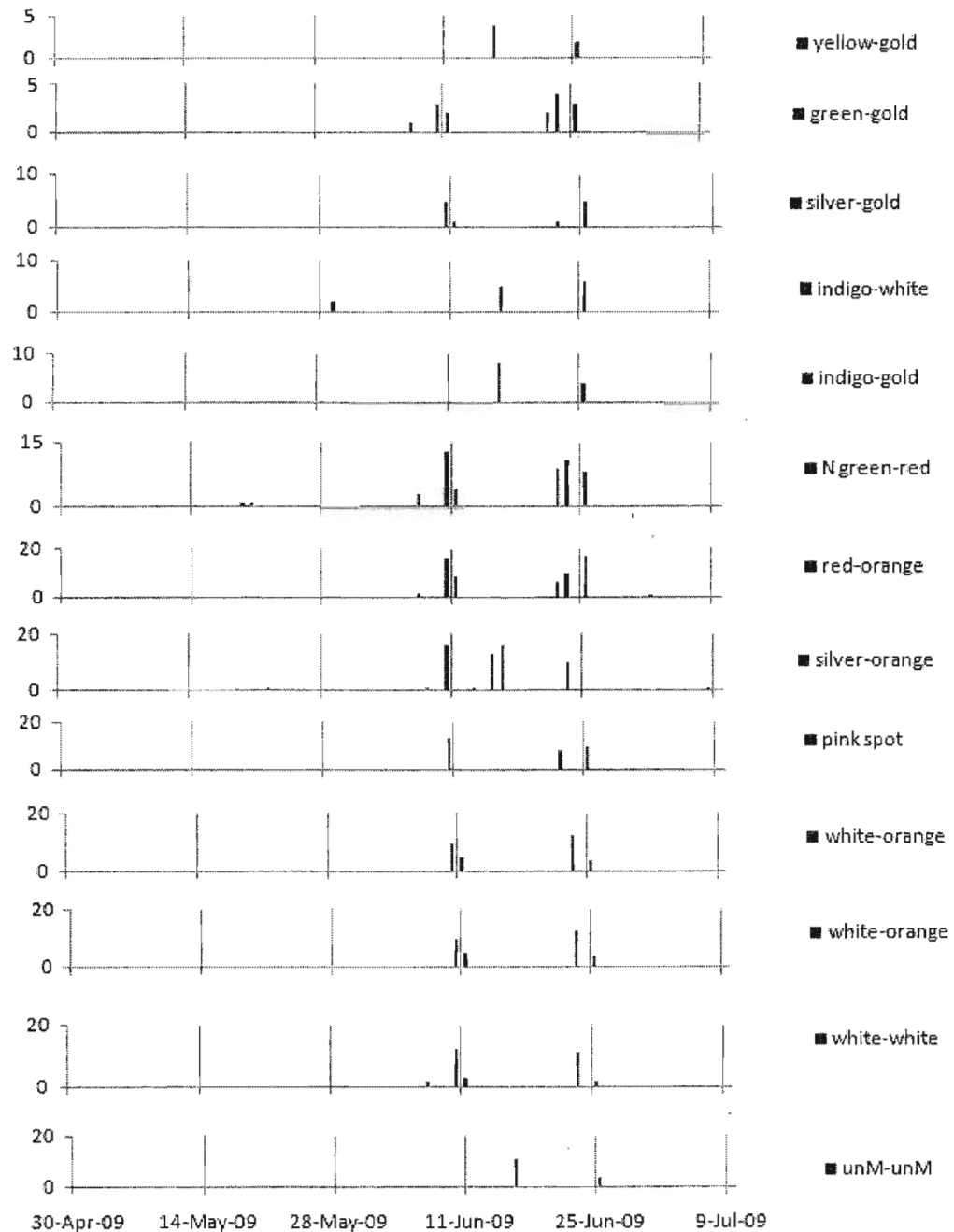


Figure 12c: Pollen foraging schedule of group C females (N=13). Group C females generally started late in the season, and collected most of their pollen in a few days of foraging. These foragers had the highest daily pollen collection rates of all the bees.

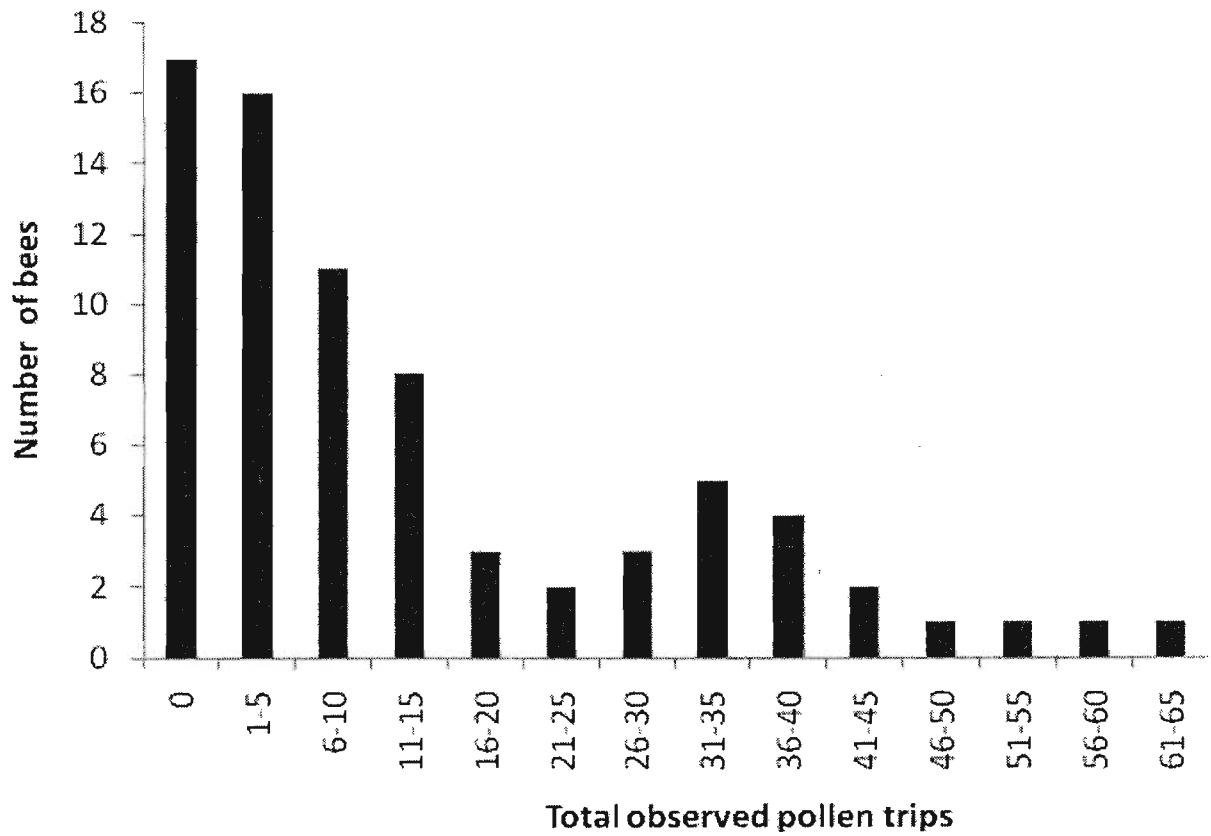


Figure 14: Variation in the observed seasonal total number of pollen trips by individual females (N=75) in 2009.

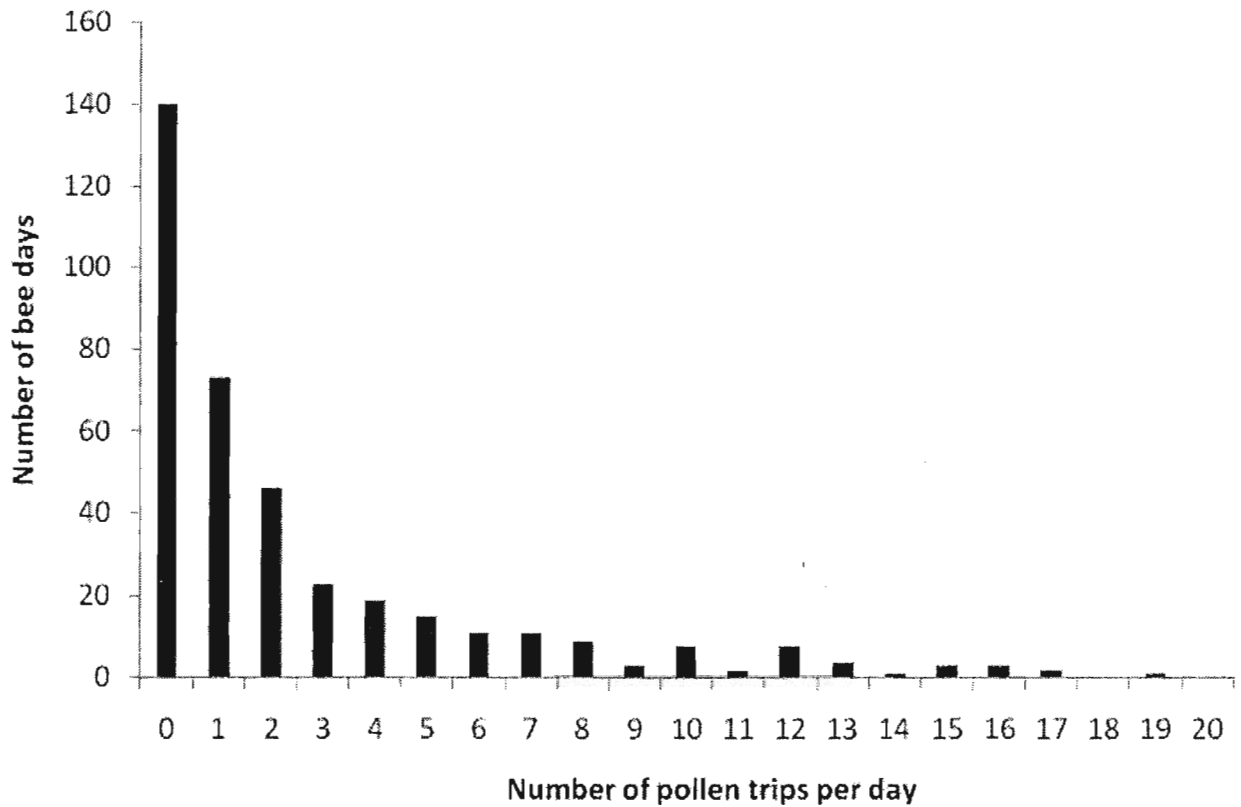


Figure 15: Variation in daily foraging activity in terms of the number of pollen trips per bee per day of bees that foraged at least once ($N=58$). A bee-day is the amount of activity performed by one bee in one day. Every individual therefore can be represented more than once.

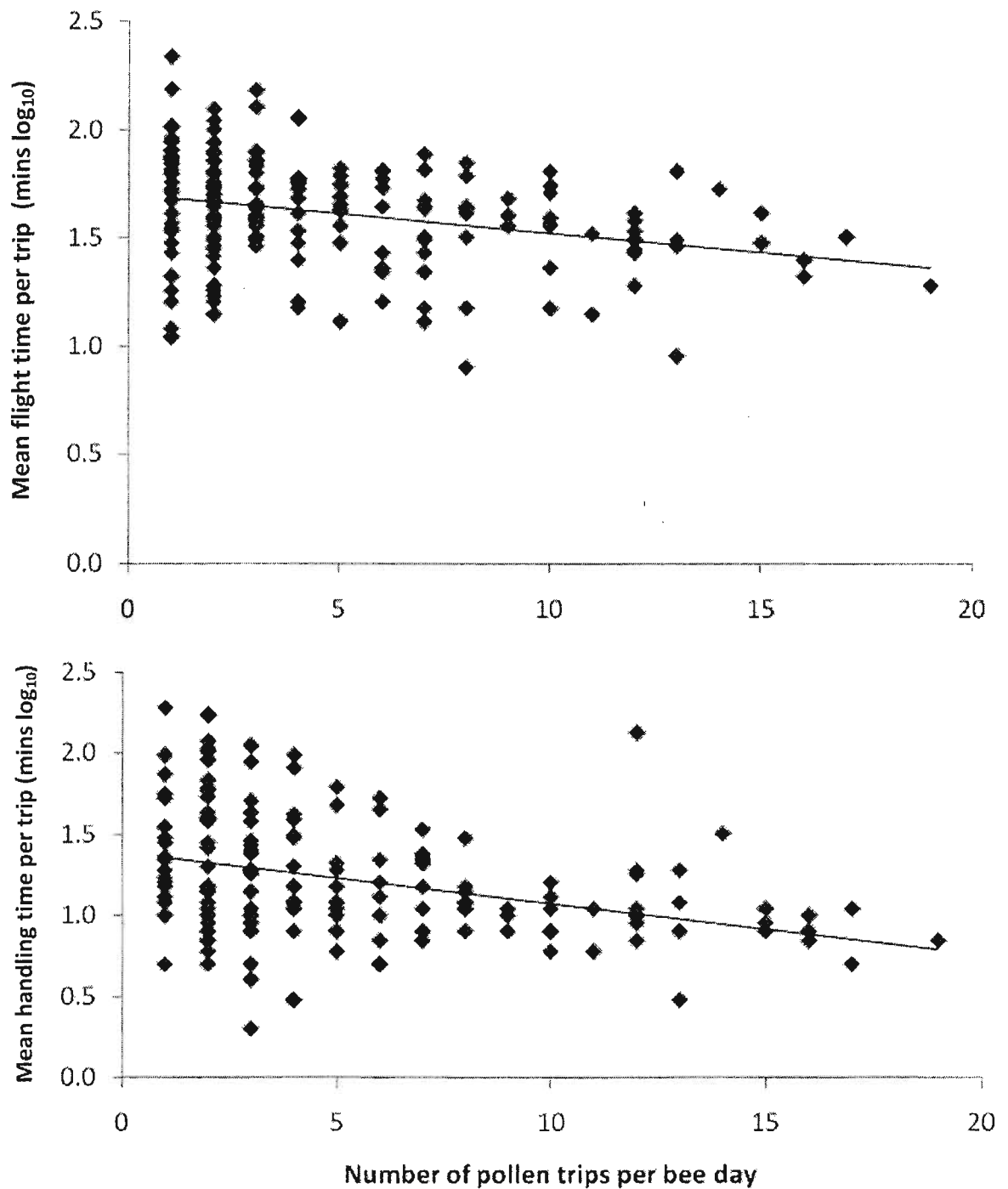


Figure 16: Relationship between number of pollen foraging trips per day and mean flight time (top) and handling time (bottom) per trip excluding non-pollen trips. As the number of pollen trips per bee day increase, the mean flight time and mean handling time per trip decreases.

Foraging consistency

Foragers showed some degree of consistency in pollen-foraging. On two separate groups of days (15 and 16 June, and 25 and 26 June) of similar temperature, light intensity and observation duration, females tended to collect the same amount of pollen on the first and second consecutive days (linear regression, $F_{(1,14)}=12.0$, $R^2=0.48$, $p=0.004$; Figure 17).

Size and foraging behaviour

Female body size was not predictive of the measures of foraging behaviour with respect to head width or IT width. Head width did not predict mean observed flight time per trip per female (ANOVA, $F_{(1,39)}=0.05$, n.s.), or mean observed handling time per trip (ANOVA, $F_{(1,39)}=1.97$, $p=0.169$; n.s. Figure 18), total observed flight time ($F_{(1,39)}=1.15$, n.s.), total observed handling time (ANOVA, $F_{(1,39)}=0.22$, n.s.; Figure 19), or total observed pollen trips per female (ANOVA, $F_{(1,39)}=0.16$, n.s.; Figure 20). IT width did not explain mean observed flight time per trip (ANOVA, $F_{(1,39)}=0.14$, n.s.), or mean observed handling time per trip (ANOVA, $F_{(1,39)}=0.01$, n.s.; Figure 18), total observed flight time (ANOVA, $F_{(1,39)}=2.29$, n.s.), total observed handling time (ANOVA, $F_{(1,39)}=1.49$, n.s.; Figure 19), or total observed pollen trips (ANOVA, $F_{(1,39)}=0.20$, n.s.; Figure 20).

It was found that non-foragers were significantly smaller than foragers with respect to IT width (ANOVA, $F_{(1,63)}=4.24$, $p=0.044$), but not head width (ANOVA, $F_{(1,63)}=1.81$, $p=0.183$; Figure 27).

Wing wear

Wing wear was related to flight activity. Specifically, wing wear accumulation was directly related to the number of observed pollen trips (linear regression, $F_{(1,23)}=8.7$, $R^2=0.27$, $p=0.007$), the total number of observed foraging trips (linear regression, $F_{(1,23)}=5.2$, $R^2=0.19$, $p=0.031$) and the observed number of flying days per bee (linear regression, $F_{(1,23)}=6.2$, $R^2=0.21$, $p=0.020$). Wing wear accumulation was not directly related to the observed number of days spent collecting pollen (linear regression, $F_{(1,23)}=3.7$, $R^2=0.14$, $p=0.066$; Figure 22). Wing wear accumulation was most explained by the number of observed pollen trips.

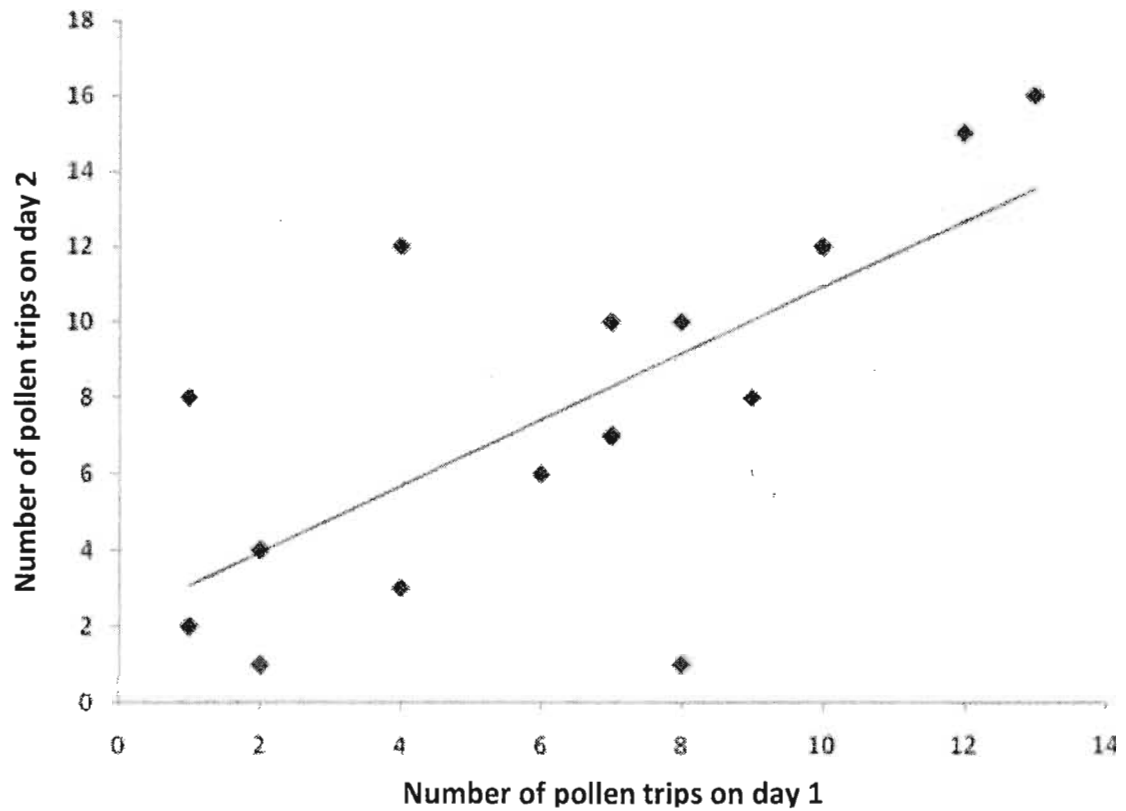


Figure 17: Number of pollen trips per day for the 15 (out of 58) foragers that foraged for two days in a row on June 15 and 16, and June 22 and 23 (N=15). Days were of similar temperature, light intensity and were observed for the same amount of time. The number of pollen trips on day 1 was correlated with the number of pollen trips on day 2.

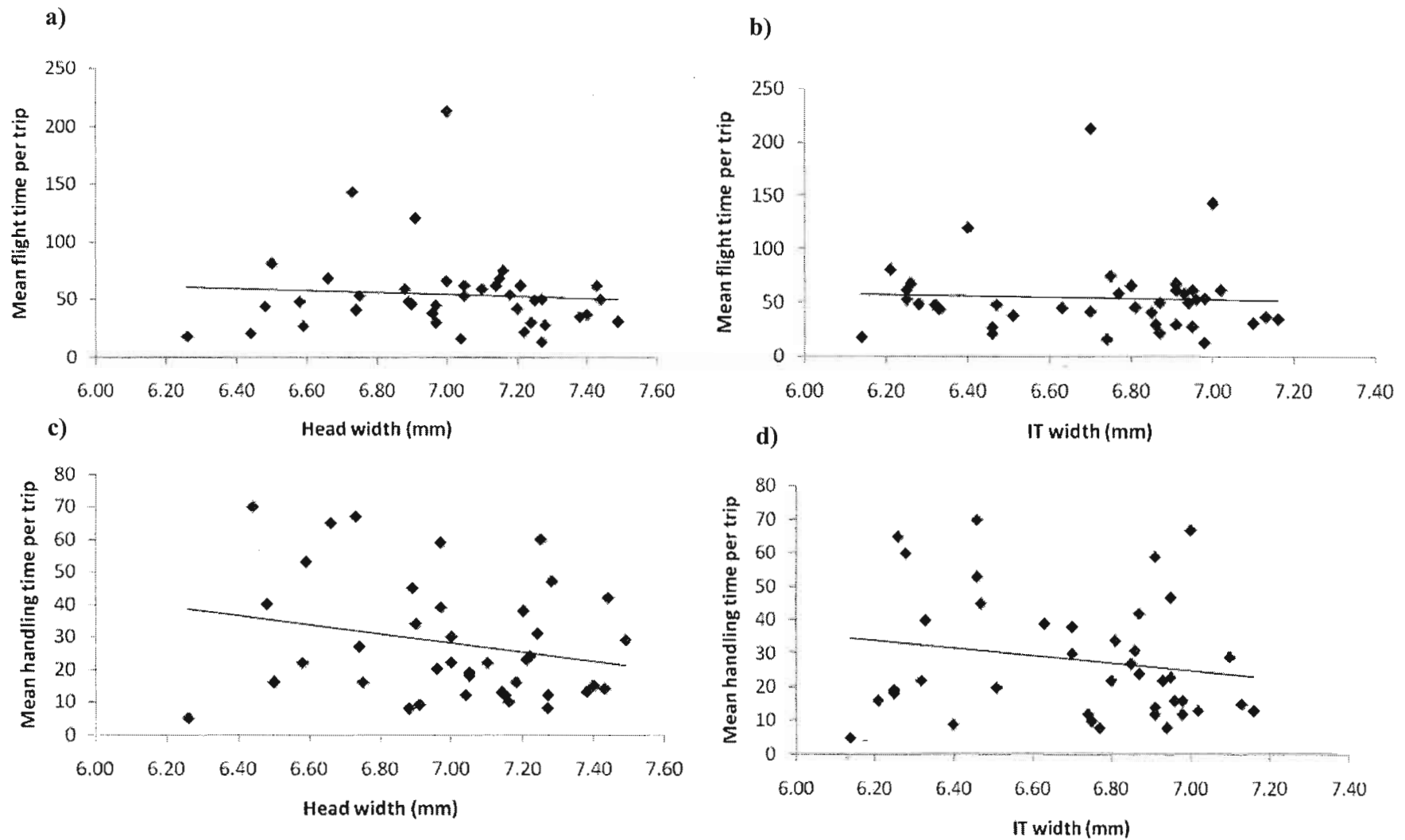


Figure 18: Relationship between body size and mean minutes of flight time per trip a), b), and handling time per trip c), d), for all 58 foragers. Body size was measured in terms of head width and IT width.

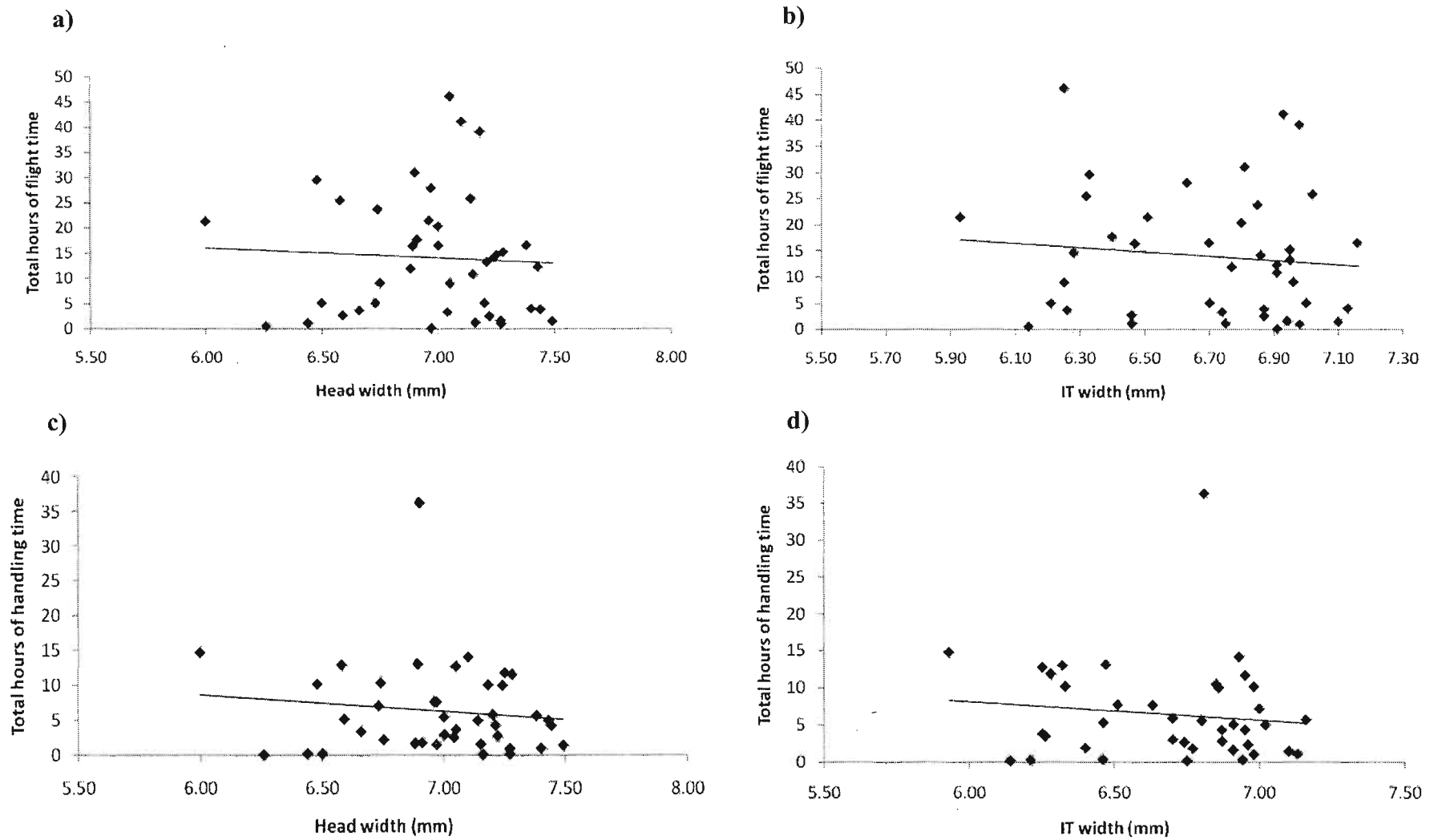
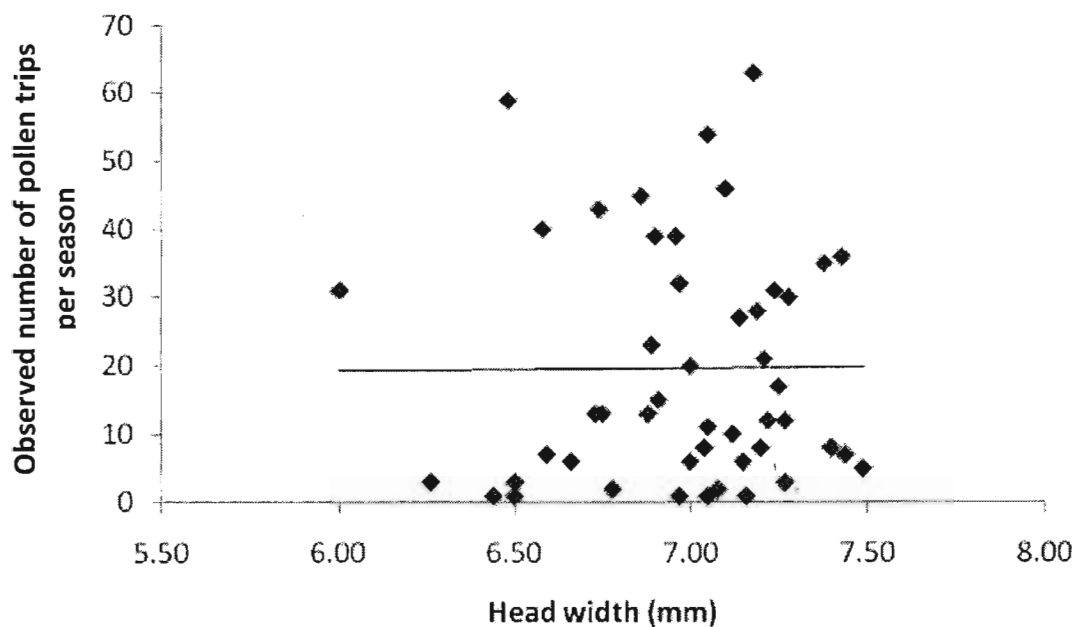


Figure 19: Relationship between body size and total hours of flight time per season **a)**, **b)**, and handling time per season **c)**, **d)**, for all 58 foragers. Body size was measured in terms of head width and IT width.

a)



b)

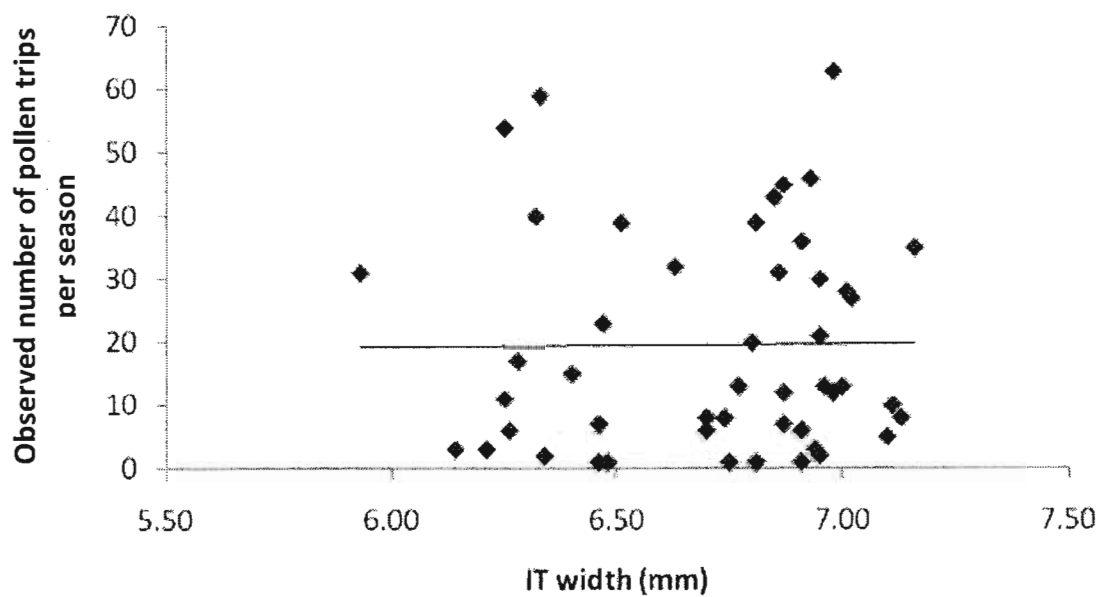


Figure 20: Relationship between body size, and the total number of pollen trips done in the season by all 58 foragers. Body size was measured in terms of a) head width and b) IT width.

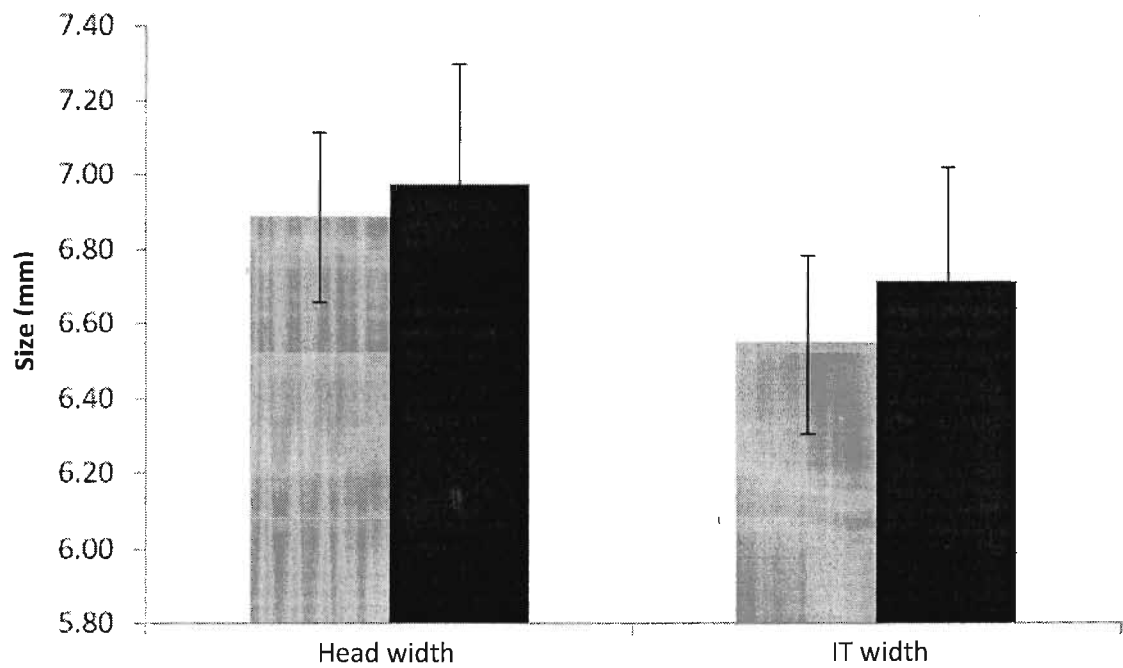


Figure 21: Differences in body size (head width and IT width \pm S.D.) of non-foragers (light) and foragers (dark). There were a total of 48 foragers and 17 non-foragers that were measured. Non-foragers were smaller than foragers in terms of IT width.

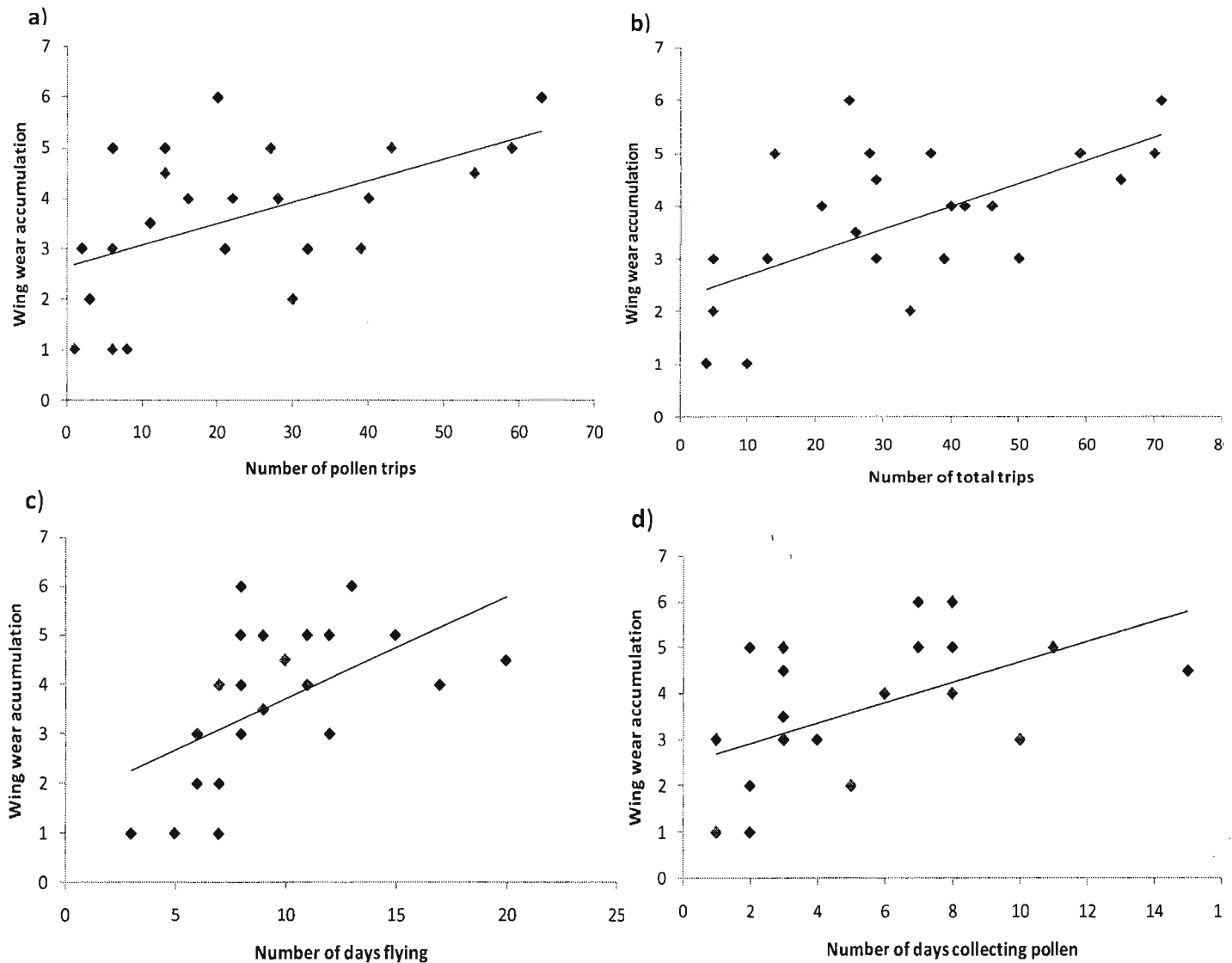


Figure 22: Seasonal accumulation of wing wear of the 25 females that had at least 2 wing wear scores, for **a)** the total number of observed pollen trips, **b)** the total number of observed foraging trips (pollen and non-pollen trips), and **c)** the total observed number of days flying and **d)** the total observed number of days collecting pollen.

Section 2 – Social implications of foraging behaviour

In 2009, there were 42 nests to which at least one pollen load was delivered; these were designated as active nests. The average nest consisted of three bees, two foragers and one non-forager. The nest foragers were observed to collect an average of 25 pollen loads per nest, and constructed and provisioned brood cells in one tunnel. Since bees were only observed for 1/3 of the season, this implies that the average nest had 75 pollen loads collected over the season. Nests had an average of 3 branches. There was a considerable amount of variation in nest occupancy, as there were two solitary nests with one forager each, 23 nests that had a single forager and at least another female, 11 nests with two foragers, and 6 nests with 3 or more foragers (Figure 23). Thus, there were 25 single-forager nests and 17 multi-forager nests.

There was no significant relationship between the number of foragers in a nest and the mean observed number of pollen trips per day per bee (linear regression, $F_{(1,62)}=1.4$, $R^2=0.0062$, n.s.), the mean observed flight time per bee per day (linear regression, $F_{(1,62)}=0.07$, $R^2=0.0203$, n.s.) or the mean observed handling time per bee per day (linear regression, $F_{(1,62)}=0.54$, $R^2=0.0007$, n.s.; Figure 24). There was also no significant relationship between the total number of females in a nest and the mean observed number of pollen trips per bee per day (linear regression, $F_{(1,62)}=2.34$, $R^2=0.0007$, n.s.), the mean observed flight time per bee per day (linear regression, $F_{(1,62)}=0.34$, $R^2=0.0028$, n.s.) or the mean observed handling time per bee per day (linear regression, $F_{(1,62)}=0.00$, $R^2<0.0001$, n.s.; Figure 25).

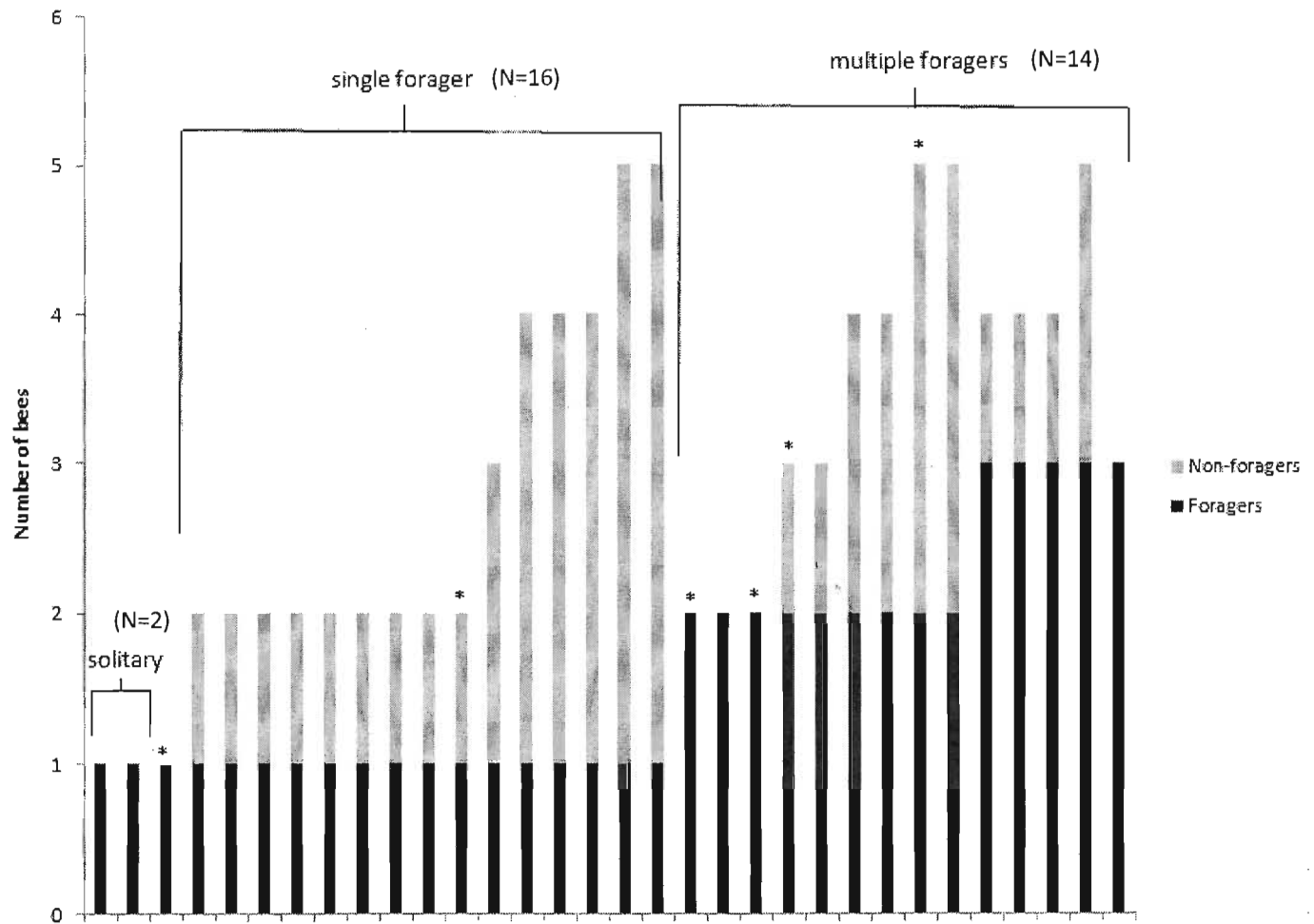


Figure 23: Numbers of foragers and non-foragers in nests of *X. virginica* (N=32). This does not include the 10 nests that could not be entered via videoprobe. Nests that could only be partially investigated via videoprobe are indicated as (*). Foragers are bees that did at least one pollen trip, while non-foragers never did a pollen trip.

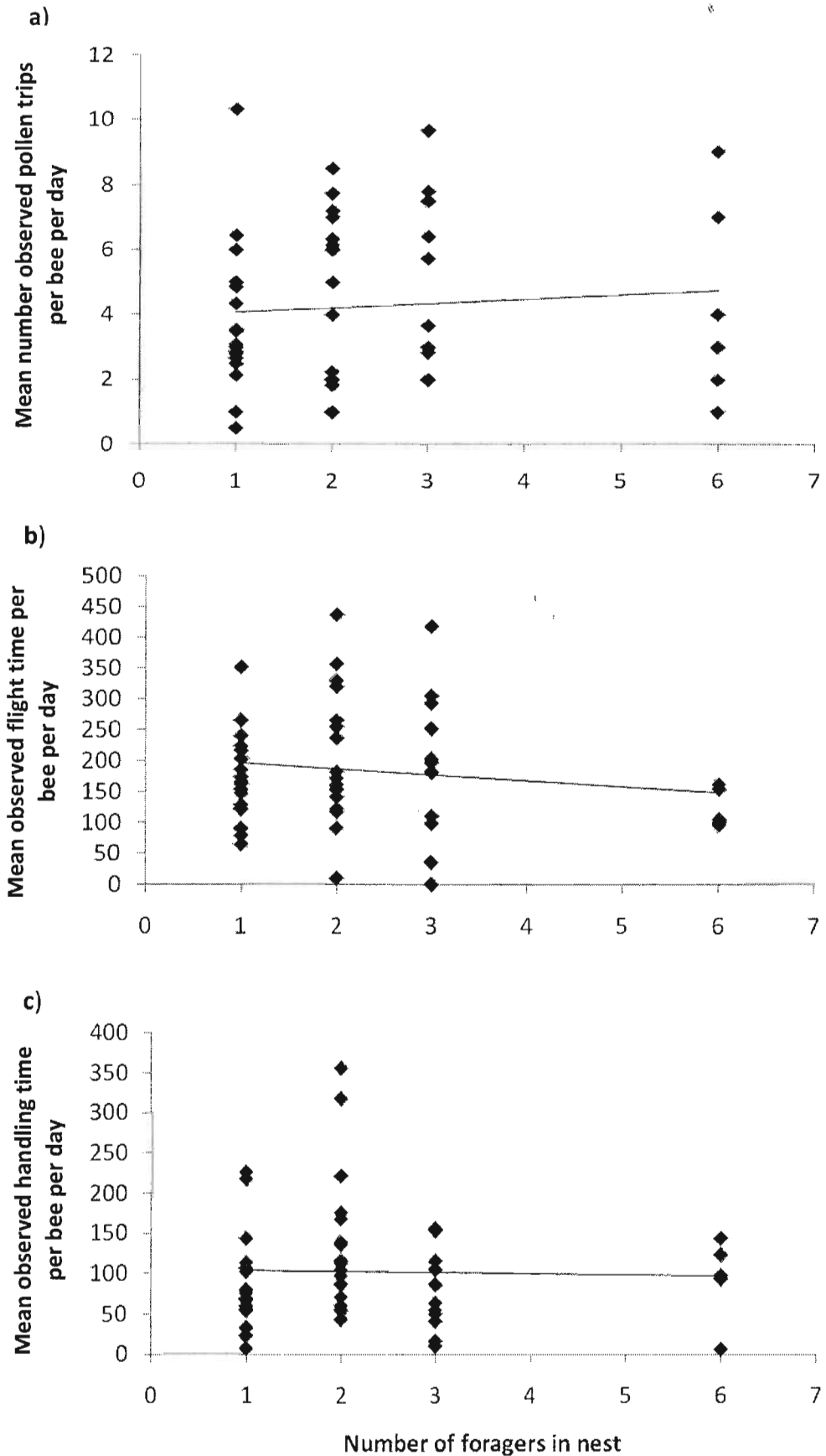
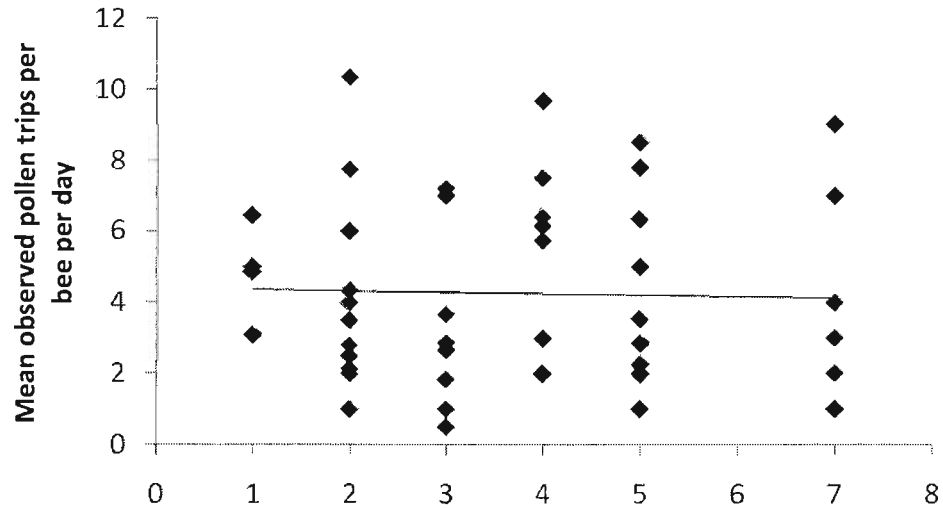
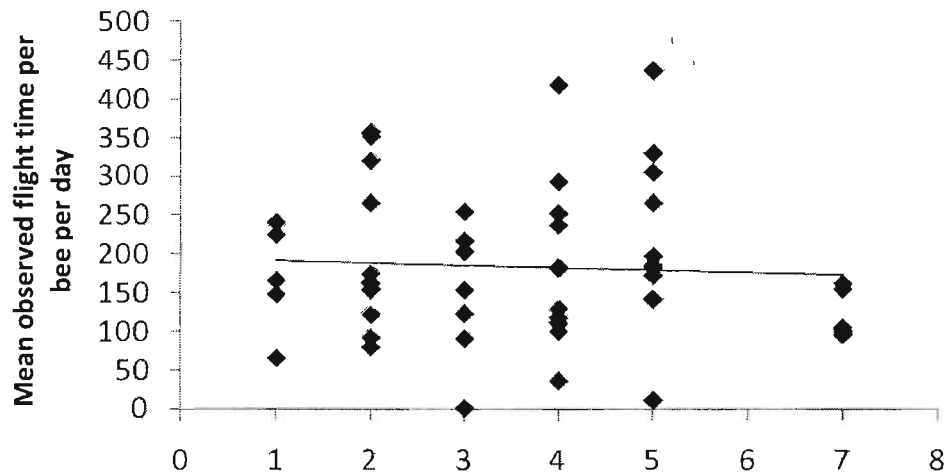


Figure 24: Per bee per nest comparison of the number of foragers and the a) mean number of observed pollen trips, b) mean hours of observed flight time and c) mean hours of observed handling time per bee per day.

a)



b)



c)

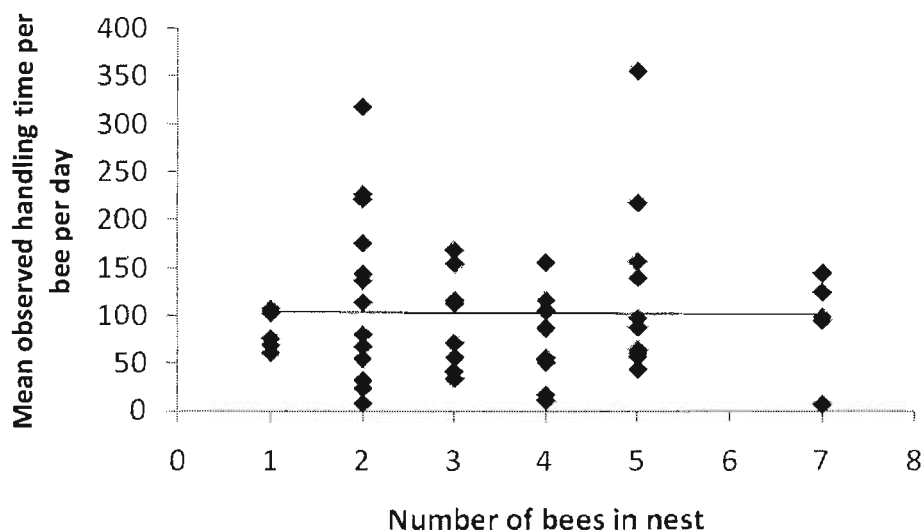


Figure 25: Per bee per nest comparison of the number of females (foragers and non-foragers) and the a) total number of observed pollen trips, b) total hours of observed flight time and c) total hours of observed handling time per bee per day.

Forager sequence within nests

In multi-forager nests, foragers were observed in some cases to forage for pollen on the same day, while at other times foragers of the same nest were observed to forage on different days. In some nests, foragers collected pollen sequentially over the season and were never seen foraging on the same day (Figure 26, Figure 27).

Within nest body size comparison of non-foragers, first foragers and subsequent foragers revealed that there was no difference in either head width (two-way nested ANOVA for which nest was controlled, $F_{(23,49)}=0.39$, n.s.) or IT width ($F_{(23,49)}=0.65$, n.s.) between any of the groups in the same nest or not (Figure 28).

First foragers started flying earlier and ended flying later in the season than subsequent foragers. Non-foragers started flying at the same time as first foragers (before subsequent foragers) and stopped flying at the same time as subsequent foragers (Table 1). Interestingly, although first foragers spent both more days both flying and foraging than subsequent foragers, there was no difference in the total observed pollen and foraging trips among first and subsequent foragers. There was also no difference among first and subsequent foragers between any of the other measures of foraging behaviour (total flight time, total handling time, total provisioning time, mean flight time, mean handling time, mean provisioning time). First foragers tended to be the largest, followed by subsequent foragers and finally non-foragers. First foragers did not accumulate more wing wear and did not have higher wing wear rates than subsequent foragers (Table 1).

Foraging behaviour and social organisation

Variables concerning foraging behaviour, body size, wing wear and important foraging dates, as discussed in the Methods section were also compared among the forager categories of (a) single forager, (b) dominant forager in a multiple-forager nest, (c) subordinate forager in a multi-forager nest and (d) non-foragers (Table 2). Dominant foragers in multi-forager nests were observed to perform more pollen trips, perform more total foraging trips (pollen and nectar), spend more days collecting pollen, stop collecting pollen later in the season, and spend more time foraging than subordinates and non-foragers in the nest (Table 2). Dominants in nests of multiple foragers were very similar to single-forager dominants with the exception that mean provisioning time per trip was higher in single-foragers. Single foragers were significantly larger than non-foragers with respect to IT width, and were the largest of all the forager categories. Subordinates were similar to non-foragers in terms of the number of days flying in the season and the day of the last flight, but were distinguished by the fact that they foraged and non-foragers did not. There were no significant differences between categories of foragers for the day of the first flight, day of the first pollen foraging trip, mean flight time per trip, mean handling time per trip, total flight time per season, head width, wing wear rate and wing wear accumulation (Table 2). There were no significant differences between any of the above measures with respect to nests, nor was there any significant interaction between nests and the foraging variables.

Table 1: The “forager order model” depicting medians and ranges of significant and nearly significant measures of observed foraging behaviours, body size and important dates with respect to the foraging order within a nest. The first females to forage are referred to as “first”, while the next female(s) to forage are referred to as “subsequent”. Differences between females were analyzed using Tukey’s test, and were denoted accordingly. Note that for non-foragers one measure was not applicable and is denoted as n.a. There was no interaction between any of the above variables and the nest

Variable	First (N = 38)	Subsequent (N = 20)	Non-foragers (N = 17)	ANOVA
Day of first flight	22 (0 – 65) _a	42 (8 – 56) _b	24 (5 – 47) _a	$F_{(2, 74)}=4.9, p=0.009$
Day of last flight	63 (24 – 70) _a	56 (24 – 70) _b	40 (20 – 70) _b	$F_{(2, 74)}=16.8, p<0.0001$
Day of first pollen – collecting flight	38 (0 – 65) _a	43 (20 – 56) _b	n.a.	$F_{(1,57)}=11.2, p=0.001$
Number of days observed flying	8 (1 – 20) _a	3 (1 – 11) _b	2 (1 – 5) _c	$F_{(2, 74)}=22.3, p<0.0001$
Number of days observed pollen – collecting	4 (1 – 15) _a	2 (1 – 16) _b	0 _c	$F_{(2,74)}=51.7, p<0.0001$
Total number of observed pollen trips	12 (1 – 63) _a	10 (1 – 43) _a	0 _b	$F_{(2,74)}=42.2, p<0.0001$
Total number of observed foraging trips (non – pollen and pollen)	19 (1 – 71) _a	16 (1 – 59) _a	2 (0 – 10) _b	$F_{(2,71)}=21.6, p<0.0001$
IT width (mm)	6.87 (6.14 – 7.16) _a	6.75 (5.93 – 6.96) _a	6.57 (6.15 – 7.03) _a	$F_{(2,63)}=2.5, p=0.0896$

Table 2: The “total resources model” depicting medians and ranges of significant measures of observed foraging behaviours, body size and important dates with respect to nests’ social organization. Single foragers were the only foragers in the nest, while dominant foragers and subordinate foragers were in a nest of more than one forager and were separated based on number of pollen trips per season, with the dominant having more pollen trips than the subordinate. Bees within nests that had a total of only one pollen trip completed in the season were not included. Differences between female classes were analyzed using Tukey’s test, and were denoted accordingly. Note that for non-foragers some measures were not applicable and were denoted as n.a. There was no interaction between any of the above variables and the nest variable.

Variable	Single forager (N=19)	Dominant forager (N=17)	Subordinate forager (N=20)	Non-forager (N=12)	ANOVA
Day of last flight	66 (29 – 70) ^a	63 (47 – 70) ^{ab}	54 (24 – 70) ^{bc}	40 (20 – 70) ^c	$F_{(3,67)}=10.5, p<0.0001$
Day of last pollen-collecting flight	56 (29 – 70) ^a	56 (44 – 70) ^a	53 (20 – 65) ^b	n.a.	$F_{(2,55)}=4.6, p=0.0135$
Number of days observed flying	8 (1 – 16) ^a	7 (2 – 20) ^a	4 (1 – 13) ^{ab}	2 (1 – 5) ^b	$F_{(3,67)}=8.4, p<0.0001$
Number of days observed pollen collecting	4 (1 – 11) ^a	4 (1 – 15) ^a	2 (1 – 7) ^b	0 ^c	$F_{(3,67)}=22.9, p<0.0001$
Total number of observed pollen trips	17 (1 – 46) ^a	30 (3 – 63) ^a	5.5 (1 – 40) ^b	0 ^c	$F_{(3,67)}=36.2, p<0.0001$
Total number of observed foraging trips (non-pollen and pollen)	25 (1 – 58) ^a	36 (10 – 71) ^a	8 (1 – 47) ^b	2(0 – 10) ^c	$F_{(3,65)}=19.5, p<0.0001$
Mean provisioning time (mins per trip)	85.5 (41 – 170) ^a	66.5 (32 – 103) ^b	73 (23 – 134) ^{ab}	n.a.	$F_{(2,46)}=3.3, p=0.0452$

Table 2 Cont'd

Mean provisioning time (mins per trip)	85.5 (41 – 170) ^a	66.5 (32 – 103) ^b	73 (23 – 134) ^{ab}	n.a.	$F_{(2,46)}=3.3, p=0.0452$
Total handling time (mins per season)	270 (16 – 6423) ^{ab}	463 (54 – 2179) ^a	169.5 (10 – 781) ^b	n.a.	$F_{(2,48)}=3.4, p=0.0410$
Total provisioning time (mins per season)	1162 (170 – 2855) ^{ab}	1742 (165 – 3234) ^a	493 (45 – 2255) ^b	n.a.	$F_{(2,47)}=4.0, p=0.0249$
IT width	6.92 (6.21 – 7.16) ^a	6.66 (5.93 – 6.98) ^{ab}	6.81 (6.14 – 7.11) ^{ab}	6.56 (6.24 – 6.87) ^b	$F_{(3,67)}=3.1, p=0.0341$

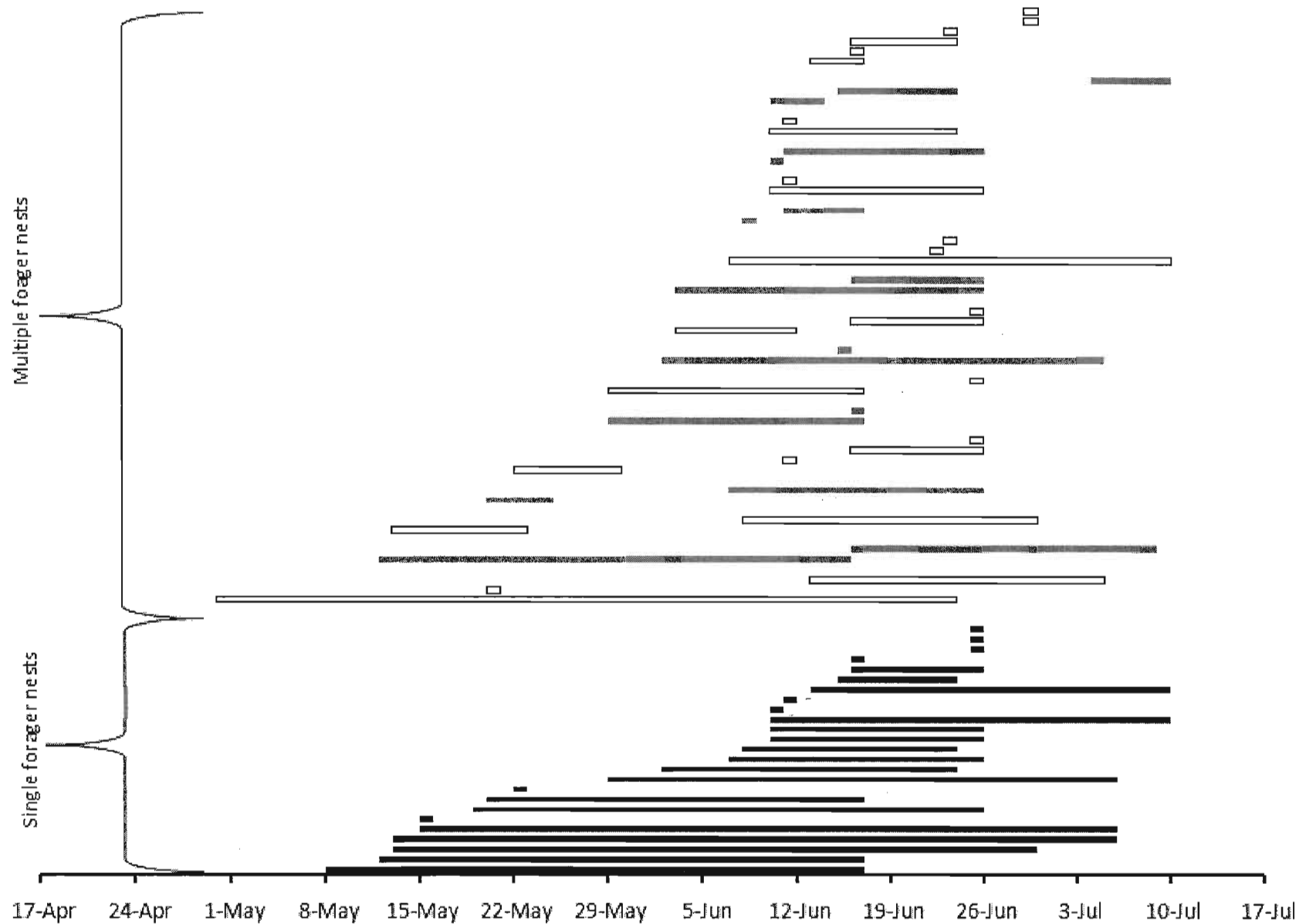


Figure 26: Foraging schedule per nest of bees that made at least one pollen trip (N=42). The foragers of multiple forager nests are coloured similarly, so that all foragers of that nest can be seen. Alternating white and gray bars of multiple forager nests were used to distinguish different nests. Every bar represents an individual, and individuals that moved between nests are included more than once.

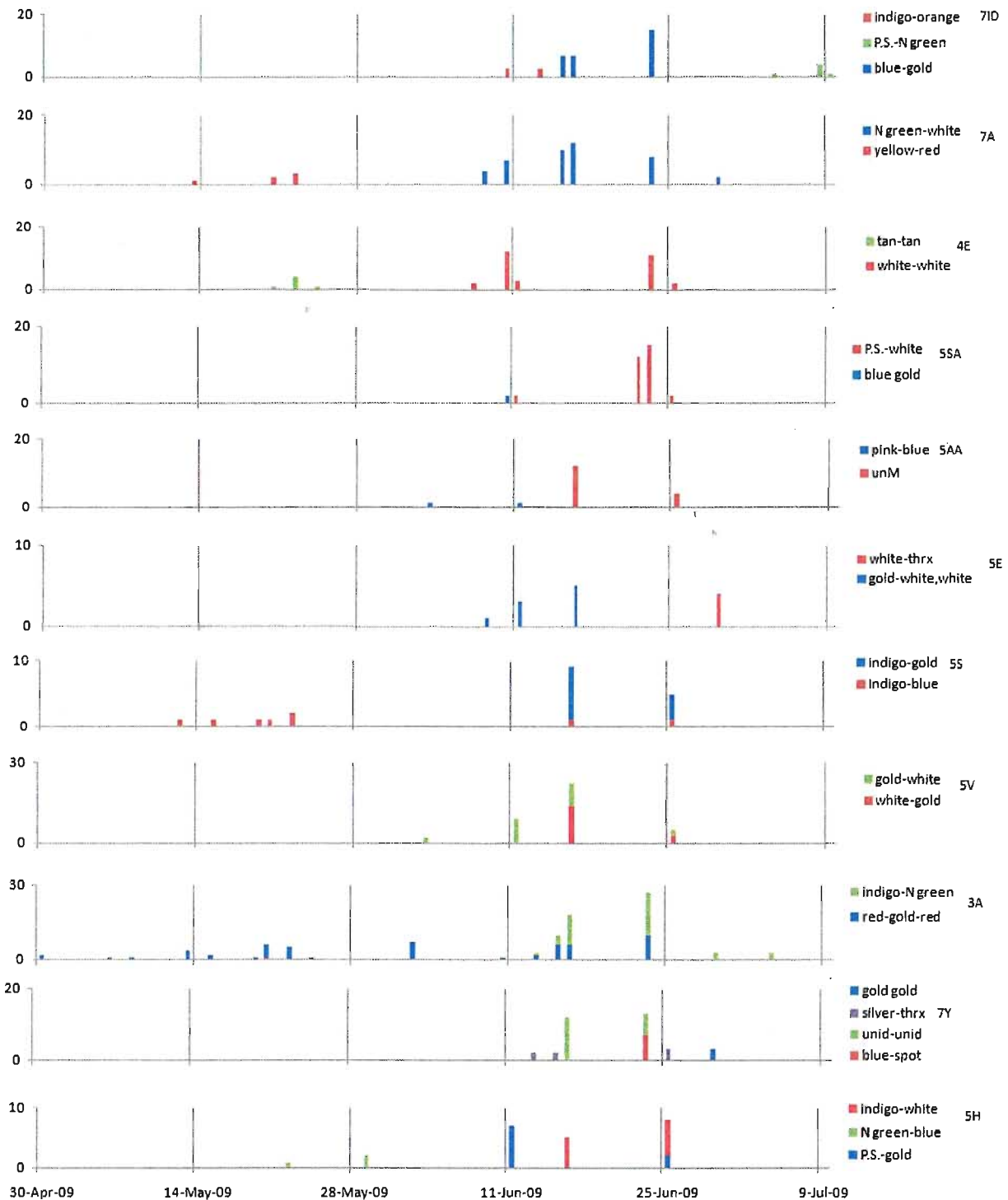


Figure 27: Pollen trip schedule of individual foragers of nests that had at least two foragers each of which did at least two pollen trips (N=11). The first six nests showed no daily pollen-trip overlap among foragers, while the last five nests displayed daily pollen -trip overlap among foragers at least once.

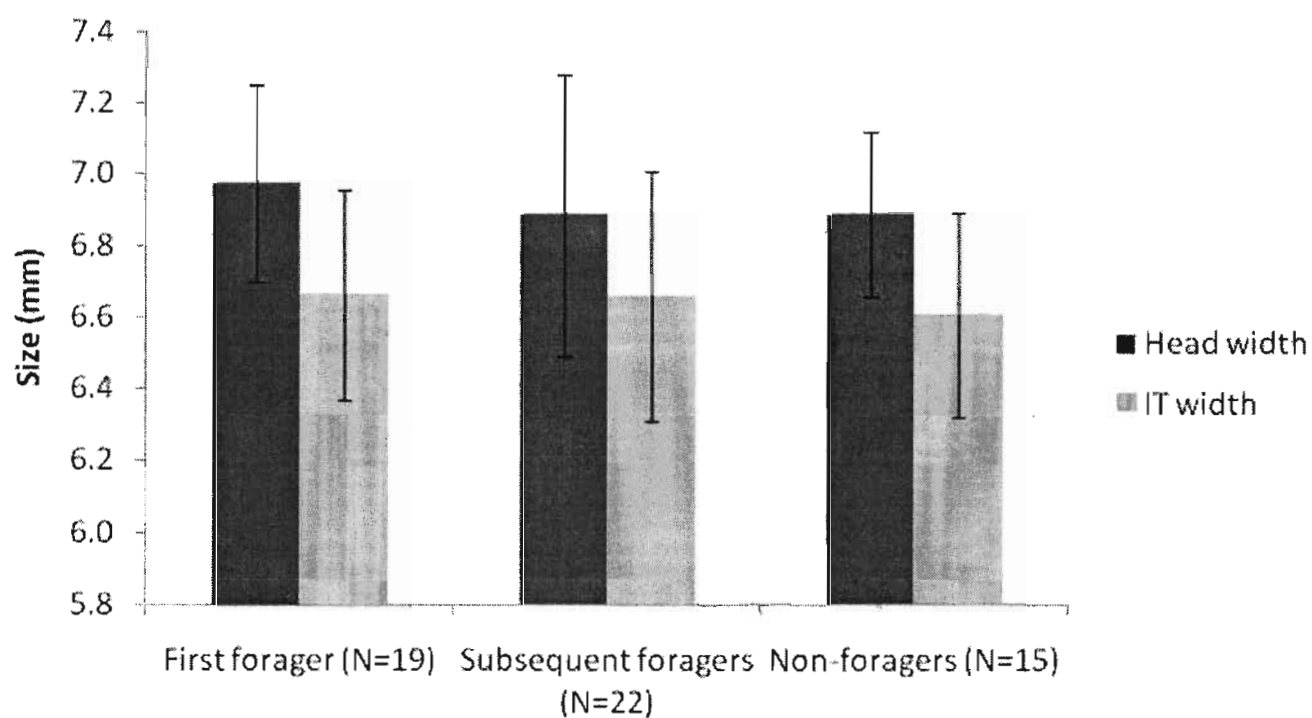


Figure 28: Average body sizes \pm S.D. of first and subsequent foragers, as well as non-foragers for which there was no interaction with the nest variable.

Movement patterns

There were 58 foragers in the 2009 population, 39 of which remained in their original nests (home bees) and 19 of which moved to another nest at least once (relocators; Figure 29), some of them moving to as many as 10 different nests (Figure 30).

Relocators were compared to home bees in terms of the measures of foraging behaviour, body size, wing wear and important foraging dates as outlined above. There were no significant differences between relocators and home bees in terms of when the bees started or stopped flying (ANOVA, $F=0.51_{(1,57)}$, n.s.; ANOVA, $F=0.08_{(1,57)}$, n.s.), started or stopped foraging (ANOVA, $F=0.10_{(1,57)}$, n.s.; ANOVA, $F=0.06_{(1,57)}$, n.s.), how many pollen trips were done in total (ANOVA, $F=1.74_{(1,57)}$, n.s.), how many total foraging trips were done (ANOVA, $F=0.91_{(1,57)}$, n.s.), or how many days were spent collecting pollen (ANOVA, $F=0.46_{(1,57)}$, n.s.). There were also no significant differences in terms of wing wear accumulation and wing wear rate between relocators and home bees (ANOVA, $F_{(1,24)} = 0.32$, n.s.; ANOVA, $F_{(1,24)} = 0.15$, n.s.; Figure 31). Females that moved the most tended to perform fewer pollen trips than those females not moving as much (linear regression, $F_{(1,66)} = 4.00$ $R^2 = 0.058$, $p = 0.049$; Figure 29). Relocators that did more than 6 pollen trips in a nest in one nest, tended to stay in these nests (Figure 29).

Figure 29: Individual flying schedule of females that made a least one pollen trip and relocated to another nest at least once, in the 2009 season. Each individuals nest movements are grouped by either grey or white bars, and each movement is denoted as a separate bar. The data labels indicate how many total pollen trips were done during an individual stay at the nest, an absent data label means that no pollen trips were done in that nest. The beginning of the bars represents the date that the female was first seen flying and the end represents when she was last seen flying. Foragers that were wanderers (visiting multiple nests a day) were excluded since it was impossible to identify all the nests these females visited.

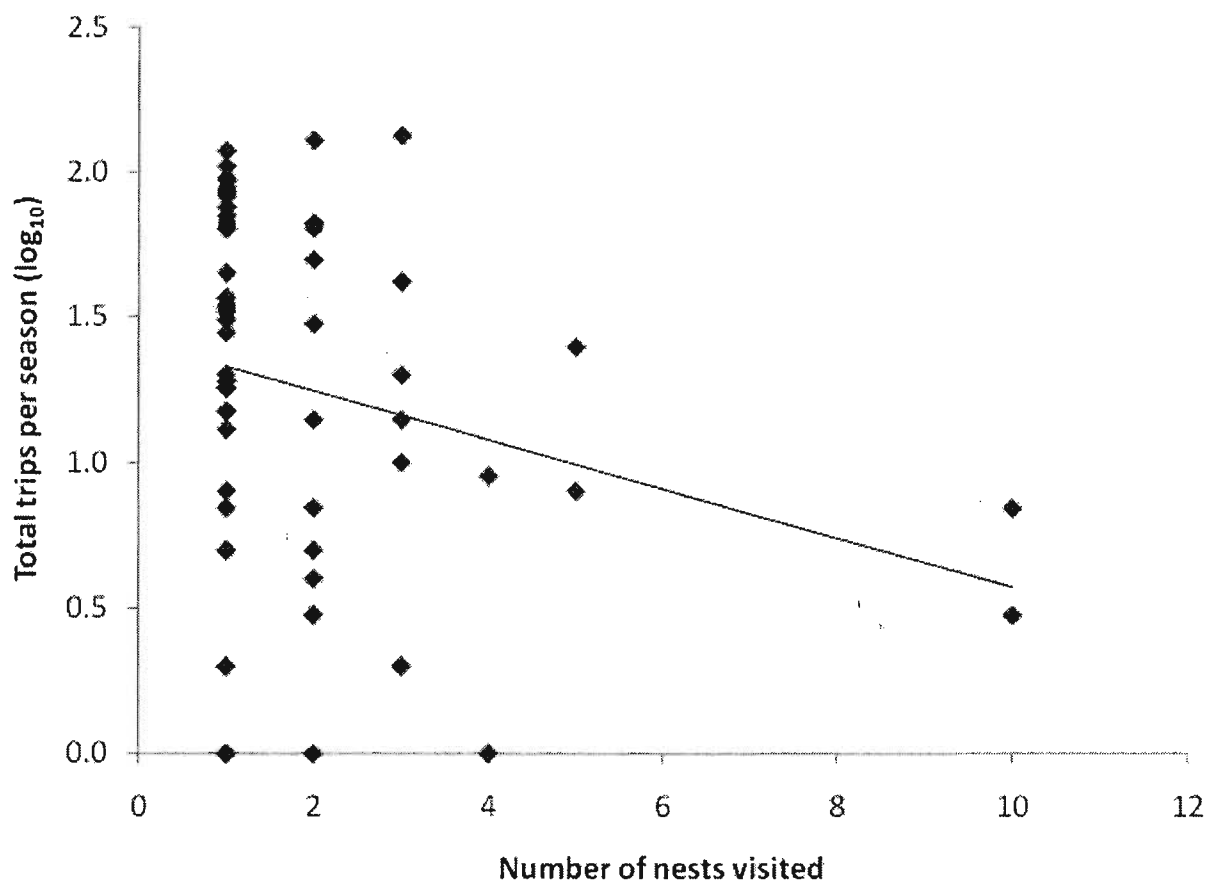


Figure 30: Number of total observed foraging trips (pollen trips and non-pollen trips) and the numbers of nests visited by all females in the population (N=75) of the 2009 season. This figure includes two bees that were observed to visit multiple nests a day (wanderers). These are represented as visiting at least 10 different nests.

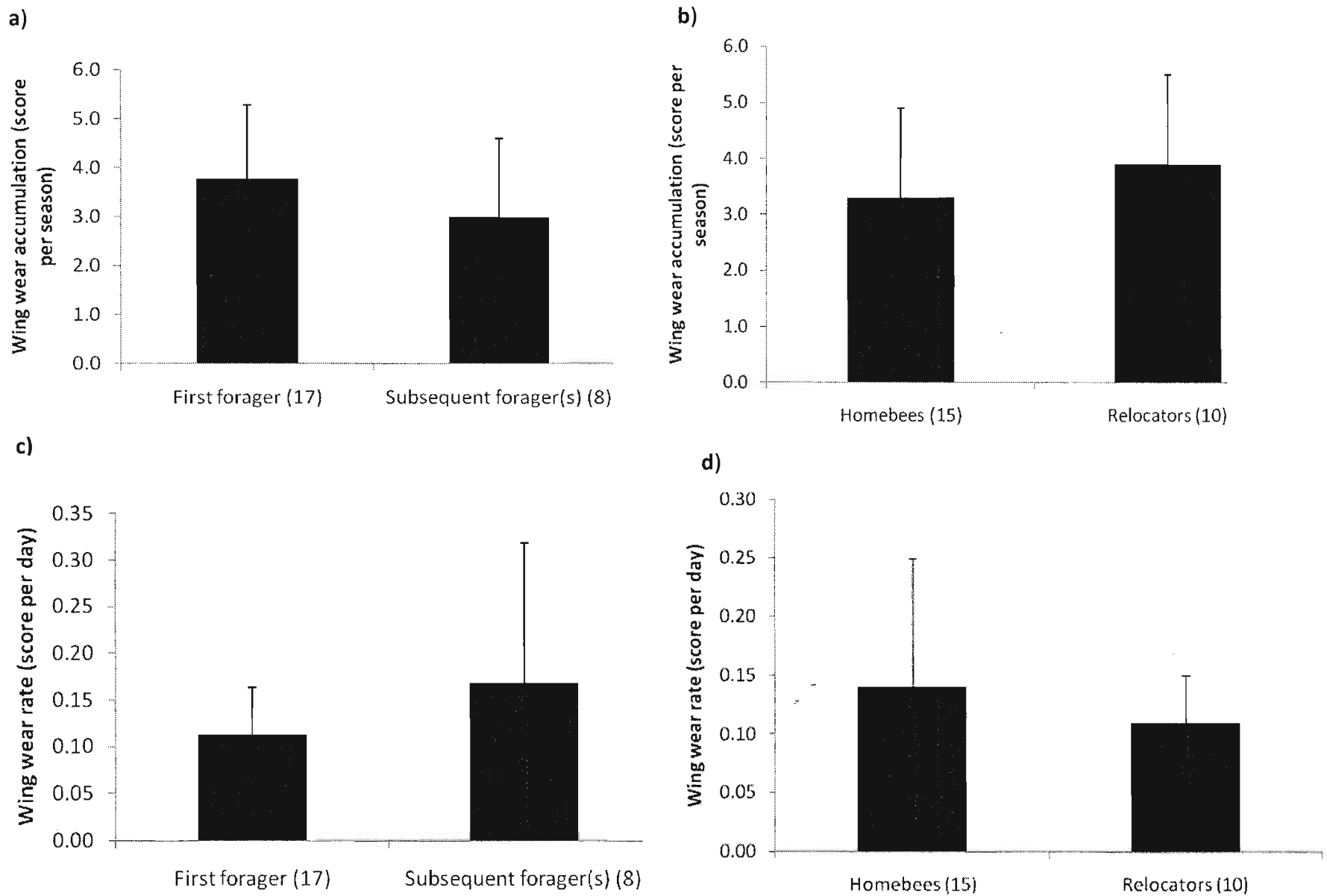


Figure 31: Wing wear accumulation \pm S.D. of **a)** first females to forage and subsequent foragers in a nest, **b)** home bees and relocators and wing wear rate (score/day) of **c)** first females to forage and subsequent foragers in a nest and **d)**, home bees and relocators. Wing wear rates and wing wear accumulation were calculated per bee per season for the 25 females that had at least 2 wing wear scores in the 2009 season.

Discussion

Section 1 – Individual forager variation in *X. virginica*

The major objective of the first section of this thesis, was to examine whether individual foragers of *X. virginica* show differences in foraging behaviour and are thus suitable for studying the social implications of brood provisioning behaviour. Large individual variation of foraging behaviour was predicted, which would arise from both the time spent foraging, as well as the number of foraging trips made. In addition, a general description of foraging in *X. virginica* was provided. Three possible foraging strategies are discussed.

Seasonality and foraging in X. virginica in Niagara

Xylocopa virginica forages in a unimodal pattern, with the peak foraging time at noon before slowly decreasing into the early evening (Figure 10), demonstrating a similar foraging pattern to *X. valga* (Wang, 2009). This pattern is in contrast to *X. pubescens* and *X. sulcatipes* which start foraging much earlier and peak in the hours of 8:00-9:00AM (Gerling and Hurd, 1983). Bees such as these must deal with much higher temperatures and only forage for about 7 hours per day. *Xylocopa virginica* in the Niagara region can forage for longer periods of time since the temperature does not reach the same extremes.

Individual females started and finished pollen collection at different times in the season (Figure 11) and foraged at varying intensities (Figure 12). There was also a large range in how many pollen trips individual females made per day or over the season (Figure 15; Figure 14), and in the amount of time that they spent foraging per day

(Appendix Figure 1). Individual rates of pollen collection were more or less consistent within foragers in some cases (Figure 12a, Figure 17), but not so consistent in others (Figure 12b, 13c). *X. virginica* foragers collected pollen on 2/3 of the days they were observed and spent 1/3 of the days not collecting any pollen (Figure 15). These results are similar to other foraging studies done with *X. pubescens*, which forage very irregularly and uniquely to the individual, often spending days on brood cell construction and preparation and, therefore, not collecting pollen (Velthuis and Gerling, 1983).

Foraging behaviour in the average bee

On average, foragers brought only three loads of pollen per day to their nests (Figure 15). This is very little, considering that *X. pubescens* requires 10 pollen trips to provision one brood cell (Velthuis and Gerling, 1982) and that many species of bees provision about one cell per day (Neff, 2008). Considering both the average pollen collection per day and that some females of *X. virginica* collected up to 19 pollen loads (Figure 15), it is likely that while *X. virginica* has the ability to provision more than one brood cell per day, the average forager likely requires more than one day to provision a cell.

There was an average of 18 observed pollen trips per forager over the entire season (Figure 14) which, given that it takes about 10 pollen trips to provision a single offspring (Velthuis and Gerling, 1982), seems very low. This would mean that an average forager in this study population only raised one to two offspring, which does not agree with Gerling and Hermann (1973) who reported an average of 6.6 offspring per *X. virginica* forager. The major reason for this discrepancy is mostly because observation

time for this study was divided among three different sites. The total pollen collection per season is likely an underestimate of what is likely the real average: $18 * 3 = 54$ pollen trips (each nest was observed about 1/3 of the time). This suggests that the average forager raised approximately five to six offspring which is much closer to the number expected from the previous study outline above.

Foraging Strategies

Females demonstrated a large degree of plasticity with respect to foraging (Figure 12). Although it is difficult to categorize the foraging behaviours observed in this population into specific strategies, the patterns of foraging that emerge seem to depend on when females started their foraging season (Figure 12).

The largest number of pollen trips performed in a day per bee were observed for bees that started later in the season (Figure 12c). Many of these trips occurred after 25 June 2009, the day on which 15 foragers ended their foraging for the season (Figure 11). This suggests the possibility that these foragers were subordinates, that were waiting for the dominants (the 15 foragers above) to finish foraging before they started collecting pollen.²

There is strong evidence that bees making more trips per day spent less time per trip both handling and flying (Figure 16). This suggests a trade-off, since in order to complete more pollen trips in a day, bees have to collect pollen faster. The obvious conclusion is that bees making more trips per day would be bringing less pollen per load,

² The link between foraging strategy and social hierarchy is the main focus of the second section of the thesis, and is discussed in more detail in Section 2.

but a study looking at foraging behaviour of 92 solitary bees, reported that the number of trips per hour was not correlated with pollen load weight (Neff, 2008). This suggests that females that make more pollen trips per day are not experiencing a trade-off, but in fact may be collecting pollen more efficiently than bees collecting less pollen in a day.

Total pollen collection per season, as well as flight time and handling time (per trip and per season) were not related to body size, as there was no relationship between these variables and either head width or IT width (Figures 19, 20, 21). This was unexpected, since size is a strong determinant of the number of trips possible in a day, and the amount of pollen carried per trip (Neff, 2008). This suggests that in *X. virginica*, size does not seem to affect an individual's ability to collect pollen.

The amount of pollen that individual bees collect seems to be related to the seasonal timing of foraging. Females that start late in the season collect less pollen than females starting early in the season (Figure 13). Late foraging females (Figures 13b, 13c) also seem to forage more intensely than early foraging females (Figure 12a) and also collect the highest amount of pollen per day.

Females that start late in the season have a disadvantage in that they would not raise as much brood as females starting earlier in the season, simply because they do not collect as much pollen as early foraging females (Figure 13). This time constraint is more of an issue in temperate carpenter bees, as tropical carpenter bees have longer foraging seasons (Gerling *et al.* 1983). Since *X. virginica* is the farthest north of any North American *Xylocopa* (Skandalis *et al.* submitted), the foraging season of this population should be the shortest experienced by any North American *Xylocopa*, which

seems to be reflected by the tendency for late foraging females to forage so intensely (Figures 13b, 13c). This pattern of increased foraging may be reflecting an act of “desperation” in order to collect enough resources to provision their young before the season is over.

Conclusions

Xylocopa virginica forages with a high degree of variation between individuals. This behavioural variation makes *X. virginica* suitable for studying foraging strategies. Larger foragers did not forage differently than smaller foragers, so size had little to no impact on various strategies within the forager group. The time at which females start to forage has an impact not only on the total amount of resources they collect, but also on their foraging strategy. Since many females of *X. virginica* live in a social group of 2-6 bees (Richards, *in press*), and since this social group is determined by a dominance hierarchy, dominants and subordinates of the same nest are very likely to forage differently with respect to each other. In the next section, the degree to which this social hierarchy impacts foraging behaviour is evaluated and discussed.

Section 2 – Social implications of foraging behaviour

In this thesis, two different models were used in order to address the major objective that different categories of females within the dominance hierarchy can be separated based on foraging behaviour. The forager sequence model was based on the order in which females started to collect pollen (Table 1), and the total resources model separated females based on the total number of observed pollen trips in the season (Table 2).

Of all the nests observed, approximately half had a single forager, while the rest had more than one forager over the season. This is in stark contrast to some of the literature that suggests that subordinates in a social *X. virginica* nest only guard (Gerling and Hermann, 1978; Hogendoorn and Velthuis, 1999). A previous study reported on the same population of *X. virginica* as in this study indicated that within a social nest there were three different kinds of females, those that had a large degree of wing and mandibular wear (primaries), those that had an intermediate amount (secondaries), and tertiaries, those that had very little wear (Richards, *in press*). Since wing wear and mandibular wear are indicative of flight activity and nest construction respectively, it was suggested that non-dominant bees within a nest may have been doing more than guarding. The present work confirmed that more than one female per nest may forage. In addition, the hypothesis that foraging behaviour can be used to separate females into three different class was supported (Tables 1, 2).

The proportion of foragers to non-foragers within the nest was much higher than in previous studies, as a foraging paper of *X. virginica* only reported one forager per nest (Gerling and Hermann, 1978). The finding that almost half of the active nests in this population had at least two foragers is therefore very significant, as it is not only possible that more than one forager of *X. virginica* can live together in the same nest, it can be common in some populations.

Within-nest social hierarchy

Considering that single forager nests raise more brood per capita than social nests (Richards, *in press*), it seems unusual that most *X. virginica* nests are social. This is an

especially perplexing issue, since based on the total resources model of dominance, single foragers in nests collect the same amount of resources as dominants in multiple forager nests (Table 2). Interestingly, it was also shown that regardless of the numbers of foragers or bees in a nest, individuals do not adjust their daily foraging behaviour (Figures 25 and 26). Since subordinates on average collect very few resources in these social nests (Table 2), these alone would likely not give much benefit to the dominant, especially because individuals of *X. virginica* do all of their own foraging and egg-laying (Gerling and Hermann, 1978). In a social nest, the dominant is likely to get some benefit from having a subordinate guard. In *Xylocopa pubescens*, it has been shown that guards can prevent pollen robbery by conspecifics and also allow the dominant forager to forage more efficiently in that she can take longer foraging trips (Hogendoorn and Velthuis, 1993). Although there was no difference in flight time between single foragers and social dominants in a social nest (Table 2), on several occasions guards did prevent other bees from entering nests, which without the guard could have resulted in pollen robbery.

From the perspective of the subordinate, there are several reasons why social nesting might be an advantage. A previous study of this population of *X. virginica* reported that nestmates were more tolerant of each other, suggesting nestmate recognition (Peso and Richards, 2010a). Since social nests of some species of *Xylocopa* often consist of related females (Hogendoorn and Velthuis, 1993), helping a related dominant nestmate could increase the inclusive fitness of the subordinate and would thus give the subordinate a staying incentive to stay within the nest. From the perspective of the dominant, a subordinate who takes over after the dominant dies, may take care of her brood and, thus, provide the dominant with an “insurance benefit” (Schwarz *et al.* 2010).

Some subordinates collected a large amount of pollen (up to 40 pollen loads, Table 2), so it is very likely that these subordinates were successful in raising brood of their own. This conclusion is strengthened by the fact that *X. virginica* create branched nests, which allows two different females to provision brood cells simultaneously in two separate tunnels, which has been observed in other *Xylocopa* and is thought to be an important part of communal nesting (Steen and Schwarz, 2000). Dominants would be unlikely to provision two tunnels simultaneously by themselves, thus leaving a nest branch for a subordinate to raise brood. Of the 29 nests that could be video-probed successfully (all tunnels could be explored), seven had two tunnels (the maximum observed) with brood cells. Of these seven nests, six had two foragers, and one nest only had a single forager. This could mean that in these six nests, each forager could have had a tunnel of brood cells. This is consistent with the video-probe observation that after the brood cells were completed, each female (when present) was seen guarding her respective tunnel (personal observations), indicating that both females were laying eggs and constructing brood cells within the same nest. This would not be unreasonable since communal nesting has been observed in other species of *Xylocopa* (Velthuis, 1987; Camillo and Garofalo, 1988, Steen and Schwarz, 2000).

Multiple foragers that are able to produce their own offspring in a communal nest would obtain both the direct fitness of raising their own offspring, and the possible added benefit of inclusive fitness from having a related nest-mate. This ideal nest situation could be even more beneficial if foragers stagger their foraging season relative to each other, which appears to be what occurs (Figure 26, 28). By doing this, one forager remains in the nest and may prevent conspecifics from entering the nests. In nests where

nestmates are related, this temporary guarding would be worthwhile for the inclusive fitness that would be obtained by allowing a related forager to forage more effectively, as discussed by Hogendoorn and Velthuis (1993). The fitness benefits from such an arrangement may be responsible in part for the conservation of social nesting behaviour in *X. virginica*.

Within-nest forager sequence

According to social theory, a subordinate that works harder in a social nest increases the indirect component of fitness, but also decreases its future survival and fecundity (Field *et al.* 2006). In some social groups, such as in the hover wasp, *Liostenogaster flavolineata*, a social queue exists in which the dominant controls all of the reproduction for a certain period of time, and there is a stable order in which a subordinate female next in line will obtain the dominant position. The subordinates, although capable of both mating and laying eggs, forego reproduction and help the dominant until she dies, at which point the subordinate becomes dominant (Field *et al.* 2006; Sumner *et al.* 2002). There is evidence that a similar temporal order of dominance is present in *X. virginica*, as evidenced by sequential foraging within the social nests. Multiple foragers within a nest tend to collect pollen at different times in the season, and in some cases there is no daily foraging overlap among females (Figures 26 and 28). It is possible that in a shared nest, females forage and provision their brood in order of dominance. The dominance hierarchy of nest-mates has been well documented in *X. virginica* (Gerling *et al.* 1989; Gerling and Hermann, 1978) although in these studies it was suggested that the dominant controls 100% of the reproduction of the nest, which I

have shown is not true (Table 1 and Figure 27). This behaviour of the subordinates to “wait” for the dominant before foraging and consequently reproducing themselves, is also demonstrated in some allodapine bees and may represent an evolutionary precursor to the worker class (Schwarz *et al.* 2010).

The order in which females forage in a multi-forager nest may be particularly important, especially since foraging start date is negatively correlated with total pollen trips performed over the season (Figure 13). When bees within a nest were split into the categories of first and subsequent foragers using the forager sequence model, it was found that although first foragers forage and fly for more days than subsequent foragers, there was no difference in the number of pollen trips performed, or in time spent foraging (Table 1). These results suggest two things. First, the foraging strategies for first and subsequent foragers are slightly different, in that first foragers forage more consistently over a long stretch of days (resembling the type ‘a’ pattern of Figure 14a), while subsequent foragers forage intensely (and possibly more efficiently on a per trip basis as suggested in Section 1, Figures 21 and 22), doing a large number of pollen trips late in the season (resembling the type ‘b’ and ‘c’ patterns of Figure 12b and Figure 12c). The tendency for subsequent foragers to forage more intensely may also explain the large degree of variation in wing wear scores in comparison to the first foragers (Figure 31). Second, and more importantly, these results suggest that subsequent foragers collected as much pollen as first foragers and, therefore, produced as much brood as first foragers as well.

The total resources model of dominance (Table 2), suggests that subordinates and dominants receive roughly equal reproductive benefit. While there is reproductive benefit in starting to forage as early as possible in the season (Figure 13), the results shown in Table 1 demonstrate that foragers do not necessarily have to be the first to forage in a nest to obtain high reproductive fitness. Under the forager sequence model (Table 1) dominant foragers collect pollen in a more consistent manner (Figure 12a) than subordinates do (Figures 13b, 13c). Although the foraging strategies are slightly different, being a subordinate forager appears to be a good strategy, in that subordinates collect a similar amount of resources as dominants, and therefore should experience similar reproductive success. This suggests a reason that may help explain the existence of social nests, especially from the perspective of the subordinate.

Body size effects and dominance

There was a significant difference in IT width between single foragers and non-foragers, and single foragers had the largest of all IT widths (Table 2). IT width has been verified as being a more accurate measurement to head size in bees (Cane, 1987; Skandalis *et al.* 2009). Head width was never significantly different among any of the categories of females that were compared.

It is possible that older females are more likely to be dominant in a nest. Although age was not possible to determine in the 2009 season, there were six females that were recaptures from 2008, and since they were marked and identified, these bees were confirmed as second-year adults. Three of the six were in social nests, and in each case they were the first to forage. This may be advantageous to foragers, especially since

the earlier that females start foraging, the more total pollen they are likely to collect (Figure 13). Although this is a very small sample size, it may be an indicator that age may influence dominance in *X. virginica*. This finding agrees with a study that shows an age-related dominance trend in *X. pubescens* (Hogendoorn and Velthuis, 1999).

Foragers vs. non-foragers

Of the 75 females that were marked and observed in 2009, there were 58 foragers and 17 non-foragers. Non-foragers flew for fewer days in the season than foragers, and individual non-foragers were never seen on more than five days, whereas some foragers were seen on 20 days (Figure 9). On 23 June 2009, non-foragers stopped flying, and few were seen again for the rest of the season. These bees either left the population or returned to a nest and did not come out again.

As predicted, non-foragers were smaller than foragers in terms of IT width (Figure 21). Since size determines the dominance hierarchy of social *Xylocopa* (Hogendoorn and Velthuis, 1999), these non-foragers were likely subordinates. Subordinate bees in *Xylocopa* serve as guards that block the entrance and prevent other conspecifics from usurping the nest (Hogendoorn and Velthuis 1993; Hogendoorn and Velthuis 1995).

It is possible that non-foraging may in fact be a kind of foraging strategy. Since *X. virginica* females can live for up to two years (Gerling *et al.* 1989), and bee flight activity is directly related to mortality (Cartar 1992), bees that are less active (non-foragers) should be more likely to survive a second year than foragers. Interestingly, the seven bees recaptured in spring 2009 that had been marked in 2008, all had wing wear

scores of one or less when first captured in 2009. Wing wear is a suitable measure of the flight activity of females, so it is very likely that these bees were in fact non-foragers in 2008. Six of these bees were foragers in their second year and interestingly, the first female to perform a pollen trip in 2009 was a second year female; she began foraging eight days before any other female. These six females were all in social nests, and in four nests, the older female was the first to forage. This suggests that these older, more experienced females were the most dominant of the nests.

Bees that wait until they are reproductively active to perform foraging activities (both foraging and egg-laying occur at the same time), delay any risks that are associated with foraging and egg-laying at separate times (Schwarz *et al.* 2010). In the case of *X. virginica*, the waiting strategy of non-foragers has been exaggerated somewhat, so after overwintering once, the female waits and does very little for the foraging season, overwinters for a second time, and then becomes reproductively active. A similar phenomenon has been observed in another carpenter bee, *Ceratina australensis* as well (Rehan *et al.* 2010).

In this study, the recaptured bees had very little wing wear, which means that in the previous year they were non-foragers. Since non-foragers were the smallest of females within the nest (Tables 1, 2), and the dominance hierarchy is in part determined by size, they were likely at the bottom of the social hierarchy. Since these bees would have to wait too long before becoming reproductive in their first season, waiting until the next year may be the only option these bees have, as being the oldest bee in the nest may

increase their chance of becoming dominant and, therefore, obtaining some reproductive fitness.

Movement patterns

In the genus *Xylocopa*, it is known that females can change nests, most notably after being evicted as a result of a dominance struggle (Hogendoorn and Leys, 1993). Bees of this genus are also known to nest in aggregations in which there are often many unoccupied nests, and they often reuse nests from year to year (Bernardino and Gaglianone, 2008; Gerling *et al.* 1989; Stark *et al.* 1990). Peso and Richards (2010b) examined relocating females in *X. virginica* in Niagara and found that half of the population changed nests. They found that there were two kinds of movements; permanent moves and temporary moves, the latter possibly being used by females to access the benefits of relocating. The present work found that 1/3 of the females in the population relocated nests. Individuals displayed anywhere from 1-10 relocations (Figure 30). The permanent movers as discussed by Peso and Richards (2010b) were evident in this study to be the females that successfully started foraging in a different nest after relocating at least once (Figure 29).

Relocators that moved repeatedly and that never stayed within a nest for long periods (Figure 29) were observed in both the present study and that of Peso and Richards (2010b). In the previous study, it was suggested that these bees could potentially be nest parasites, searching for available nests in which to lay their eggs. Since some of these bees were observed to bring pollen to a nest in some cases (Figure 29), it is unlikely that they were social parasites, as bringing a pollen load to a nest that was intended to be

parasitized would logically be a waste of energy to a nest parasite. A more likely hypothesis is that these relocators were attempting to become nest residents and to find reproductive opportunities. These relocators were likely expelled from the nest by the resident females after bringing a pollen load, before moving on to look for nest space elsewhere.

Some bees that changed nests foraged less at the initial or second nests, but later concentrated foraging effort in one nest after several relocations (Figure 29). This indicates that these females were searching for suitable nests in which to provision their brood (Peso and Richards, 2010b) and eventually were successful. These suitable nests seem to be rare, which is evident from the fact that such a large proportion of the population are relocators. One female's behaviour also indicates that social nesting may be preferred. This particular female was first seen coming out of a nest on 8 June 2009 and then again coming out of a different nest on 10 June. On 10 June, she left the nest at 9:00 in the morning and spent all day going in and out of any nest she reached. Finally at 4:20 in the afternoon, she started digging a new nest. She finished digging on 13 June, and on that day another female had moved in to the newly dug nest. The female that dug the nest then started foraging from this day forth carrying out a total of 13 observed pollen trips. The additional female stayed for the rest of the season and never foraged, so she was very likely a nest guard. One tunnel of the two in the nest had brood cells which was confirmed by observations with the video-probe.

The fact that the above observed bee dug a nest in a bench with a large number of active nests indicates that *X. virginica* may have a strong preference for nesting in

aggregations. Since foundresses gain certain fitness advantages from having a nest guard (Hogendoorn and Velthuis, 1995, Hogendoorn and Velthuis, 1993), this preference may explain why this particular bee dug a nest in a dense aggregation and why she was not aggressive when another female moved in. This social nesting preference also may explain why so few solitary nests were observed. This has also been demonstrated in *Exoneura bicolour*, in which experimenters created a surplus of nests in a substrate during a time at which foundress dispersal was common. There was no increase in independent nesting when there were more available nests, indicating that cooperative nesting was a preferred strategy (Bull and Schwarz, 1996).

There were no differences in any of the variables tested among “home bees” that never relocated and “relocators” that relocated at least once. This was a surprising result, since bees that changed nests were expected to have lower reproductive fitness than bees that did not have to move. Although home bees and relocators were similar, the negative correlation between the number of nest movements and the total number of pollen trips (Figure 30) strongly suggests that although possible, successfully relocating to other nests and producing brood is risky. Subordinate females may achieve more reproductive benefit by waiting to raise their own brood in the nest of the dominant or by helping the dominant to raise its brood (if they are related), than by trying to relocate to a new nest and raise their own brood elsewhere. If social dominance within the nest is controlled by forager order, then subordinates that are of lower ranks than other subordinates, may be more likely to leave since they will have to wait too long to provision any number of brood. The individual ‘P.S.- green’ of the first nest from the top of Figure 27, serves as an extreme example of how long a third forager may have to wait before foraging can

commence. Since waiting too long is a possibility, social nesting for the subordinate may not be favourable, but it may be better than the alternative of trying to establish a nest independently (Richards, *in press*).

General Conclusions

The major objective that foraging behaviour can be used to separate females into categories was supported, although it was found that the forager sequence model (separating females according to the order in which they foraged) explains the fitness benefits of being a subordinate better than the total resources model. When females were separated in the former model, first foragers were larger (although not significantly) than subsequent foragers, which was predicted, but first foragers collected as many resources as subsequent foragers which was not predicted. The hypothesis that the order of largest bees to smallest bees is dominants (first forager), subordinates (subsequent foragers), and non-foragers was supported in both models in terms of IT width. The hypothesis that females that never move should forage more and be larger (and, therefore, produce more brood) than relocators, was not supported. Both relocators and home bees were of similar size and foraged no differently than one another.

Future studies regarding the sociality of both *X. virginica* and other species of *Xylocopa* will likely reveal a more complicated and time-dependent version of social organization. The degree of detail in these studies is extremely important, since it is obvious that essential observations were missed in a previous study of *X. virginica* (Gerling and Hermann, 1978). Studies of the social organisation of *X. pubescens*, *X. suspecta*, *X. frontalis* and *X. grisescens* found that many kinds of sociality occur after a

daughter reactivates her mother's nest (Velthuis 1987; Camillo and Garofalo, 1988). In all the above species, multiple foragers were observed as well as communal, quasisocial and semisocial forms of sociality (Velthuis 1987; Camillo and Garofalo, 1988). Division of labour has been demonstrated in *Xylocopa sulcatipes* as some nests have both a forager and an egg-layer (Stark, 1990). Examples from these species illustrate that intensive observation of *Xylocopa* behaviour is essential if the degree of sociality is to be addressed, since observations of sociality depend strongly on when the nest is watched in the season. The findings of the present study seem to suggest that *X. virginica* are capable of more social plasticity than previously thought, and thus are more similar to other bees of the genus *Xylocopa*.

It was discussed earlier that communal nests may represent an ideal nesting strategy that may partly explain the continued existence of social nests of *X. virginica*. Future studies in which within-nest observations can be done would be ideal, since reproductive fitness of different nests could be estimated more appropriately, in addition to being able to use behavioural observations to confirm the different kinds of social nests that are very likely to be present in *X. virginica*. While large groups are unlikely to have more brood fitness per capita than solitary nests (Richards, *in press*; Figures 25, 26), nests which consist of multiple foragers which are able to raise brood should be studied in more detail, in order to address the hypothesis above. Dissections of such nests would be helpful as the number of brood produced (as a measure of reproductive fitness) within a nest could be known rather than estimated.

Xylocopa virginica forages in a variety of different ways and has proved to be an important bee in studying foraging strategies. The individual variations or “strategies” that females use seem to be very dependent on the time that females start to forage in the season. The present work shows that not only can there be more than one forager in a social nest, but that the dominance order within a nest may depend on forager order, as the first bees foraging tend to collect more total pollen than the later foragers. This finding challenges previous literature (Gerling and Hermann, 1978) which suggested that only the dominant forages while the subordinates do nothing, which was proven to be untrue. Another interesting discovery is that older bees that had very limited activity in the first season, became foragers in the second season. This implies that waiting for the next year may be a way of becoming a dominant forager, which is consistent with findings that age is a determinant of dominance in the *Xylocopa* genus.

Foraging behaviour within *X. virginica* depends on a unique interaction between the needs of the individual forager and social organisation in nests with more than one bee. This becomes clear when individual foraging patterns are examined from an individual perspective within social nests. Since many bees of this genus have so many options with respect to how they forage, when they forage, or whether or not to live socially, further research should be directed to not only this species but *Xylocopa* as a whole, on any question regarding social evolution, foraging strategy and their interaction.

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Appendix

Table 1: Normality of measures of foraging behaviour and size per bee for all 75 bees of the 2009 season.

Variable	Normality	p value (Kolmogorov-Smirnov)
Day of first flight	Non-normal	$p < 0.0100$
Day of last flight	Non-normal	$p < 0.0100$
Day of first pollen-collection flight	Non-normal	$p < 0.0100$
Day of last pollen-collection flight	Non-normal	$p < 0.0100$
Number of days observed flying	Non-normal	$p < 0.0100$
Number of days observed pollen collecting	Non-normal	$p < 0.0100$
Total number of observed pollen trips	Non-normal	$p < 0.0100$
Total number of observed foraging trips (pollen and non-pollen)	Non-normal	$p < 0.0100$
Mean flight time (mins per trip)	Non-normal	$p < 0.0100$
Mean handling time (mins per trip)	Non-normal	$p < 0.0100$
Mean provision time (mins per trip)	Normal	$p = 0.1035$
Total flight time (mins per season)	Non-normal	$p < 0.0100$
Total handling time (mins per season)	Non-normal	$p < 0.0100$
Total provisioning time (mins per season)	Non-normal	$p < 0.0100$
Wing wear accumulation score	Normal	$p > 0.1500$
Wing wear rate (score per day)	Non-normal	$p < 0.0100$
Head width	Normal	$p > 0.1500$
IT width	Non normal	$p < 0.0100$

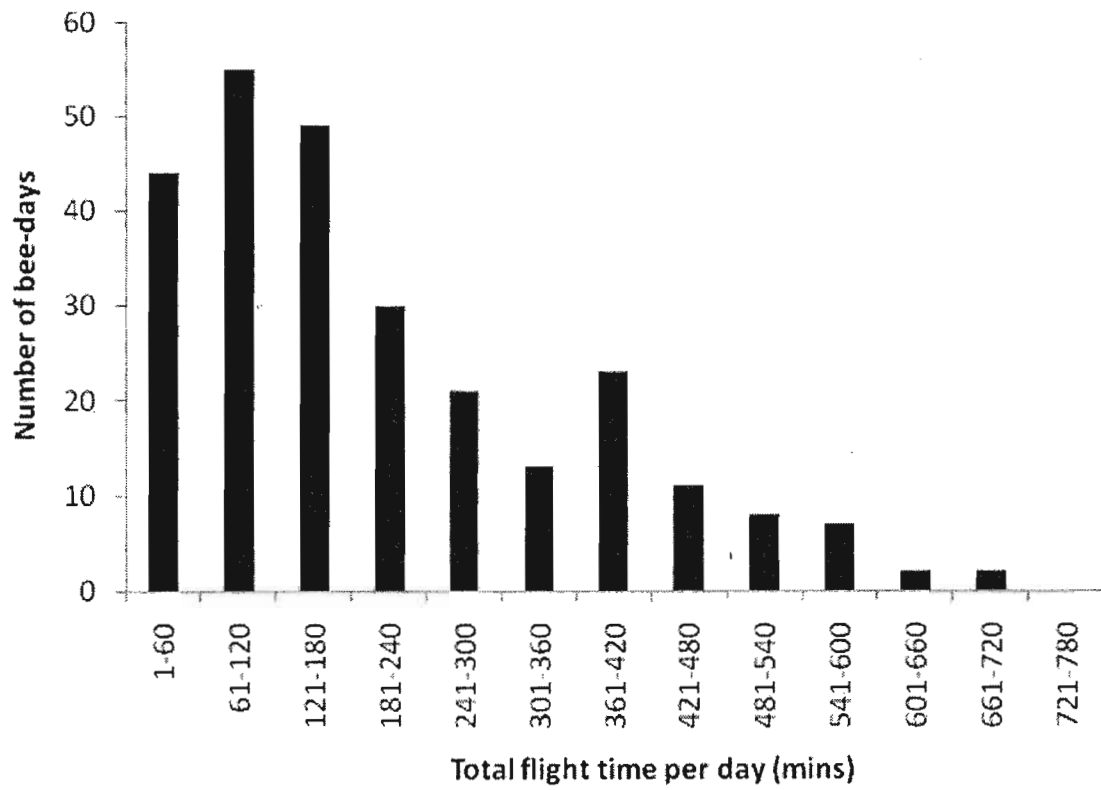


Figure 1: Mean total flight time per bee per day in minutes for all bees that collected pollen at least once (N=58).

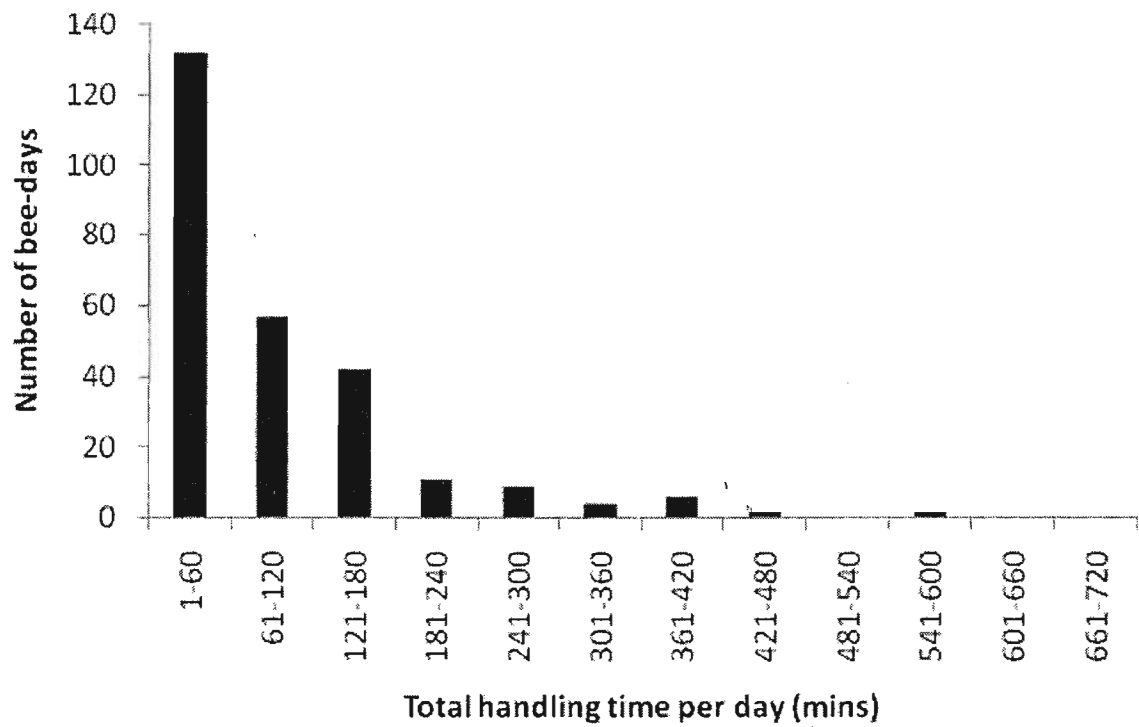


Figure 2 : Mean total handling time per bee per day in minutes for females that collected pollen at least once (N=58).

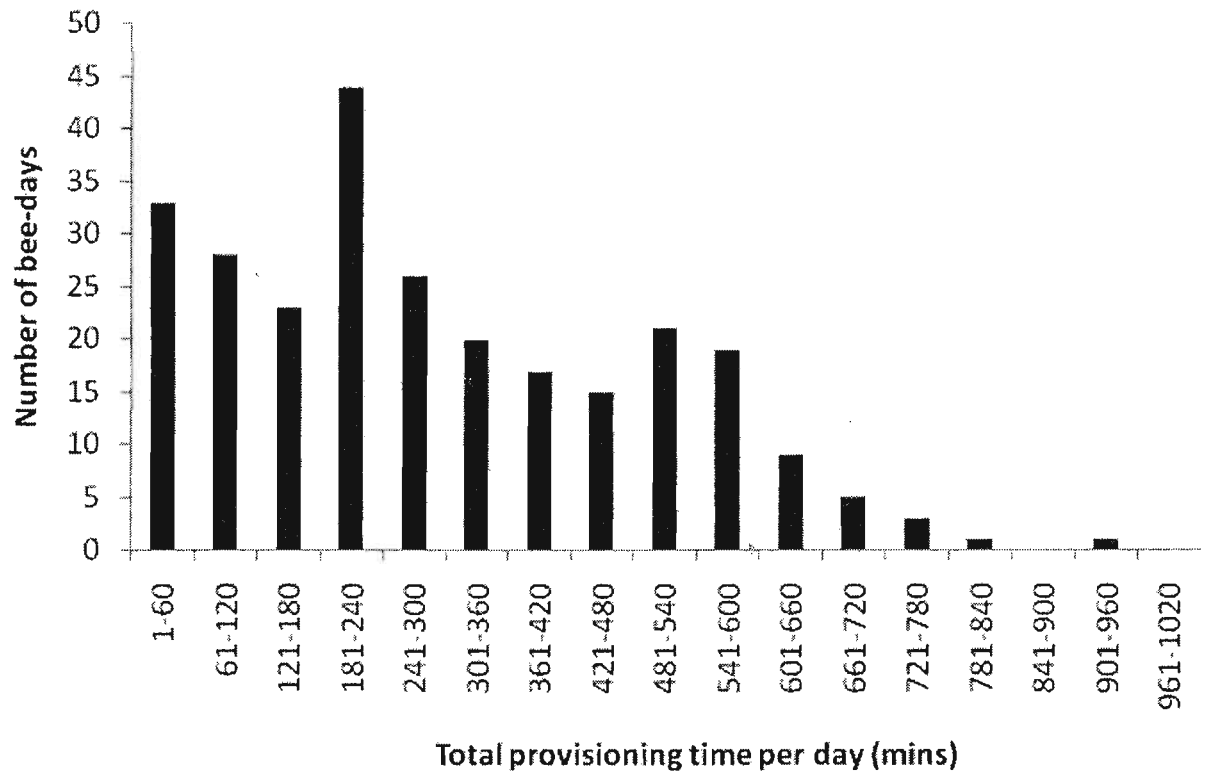


Figure 3: Mean total provisioning time per bee per day in minutes for females that collected pollen at least once (N=58).